

Coronary Risk Factor Modification

Influence of physical exercise and
smoking cessation on plasma HDL

Ingo Stubbe



Lund 1982





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**Influence of physical exercise and
smoking cessation on plasma HDL**

Ingo Stubbe



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- III. Stubbe I, Hansson P, Gustafson A, Nilsson-Ehle P: Plasma lipoproteins and lipolytic enzyme activities during endurance training in sedentary men: changes in HDL subfractions and composition. Accepted for publication in Metabolism.
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to Sebastian, Gabriel, Carita
and the prosperity of coming
generations.

BACKGROUND

CHD - the *killer number one*

Coronary heart disease (CHD), generally due to atherosclerosis of the coronary arterial bed, is the most common cause of death in this country (1) as well as in many other industrialized nations. The incidence of CHD in Sweden is among the highest in Europe and is still increasing (2), annually causing society great economic losses.

Coronary risk factors

During the past twenty-five years numerous large-scale epidemiological studies have pointed out three factors consistently and independently associated with a highly increased incidence of CHD. These major coronary risk factors are cigarette smoking, elevated blood pressure and elevated serum cholesterol levels (for reviews see 3, 4, 5). A significantly greater risk for CHD is also found in a number of metabolic diseases, such as chronic renal failure (6) and particularly diabetes mellitus (7).

Also, a number of personal attributes and environmental factors are over-represented in populations with increased CHD morbidity. These less well-established risk factors are a diet with a high content of saturated fat and cholesterol (4, 8, 9), sedentary lifestyle (10, 11),

the use of oral contraceptives (12) and psychosocial factors (13, 14). Moreover, other factors such as age, sex, and heredity, are also important determinants for CHD, although they are of little interest from the therapeutic point of view.

Epidemiological investigations and clinical observations have demonstrated close relationships between most of the coronary risk factors. For example obesity, never proven to be an independent risk factor, contributes to CHD risk through blood pressure elevation (15) and disturbances in carbohydrate and lipid metabolism (16). Moreover, elevated levels of serum cholesterol are associated with many of the other risk factors, such as cigarette smoking (17), sedentary lifestyle (18), a *rich* diet (4, 8), and oral contraceptives (19). Elevated levels of serum triglycerides have also been pointed out as an indicator for CHD risk (20), but never been proven to be an independent risk factor (21).

Serum cholesterol is not a metabolic entity. In the blood stream cholesterol circulates mainly in two types of lipoprotein particles with entirely different functions, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (see below). From studies using separate measurements of LDL and HDL it is clear that the increased risk for CHD can be entirely ascribed to increased LDL cholesterol levels (22, 23, 24). In contrast, high concentrations of HDL cholesterol are associated with a decreased incidence of CHD, and during the last decade HDL cholesterol has been established as a strong *negative risk factor*.

A reduction in HDL in atherosclerosis was observed by

Barr already in the early fifties (25). This finding was confirmed in several later studies (26, 27), but large-scale population studies were hampered by the lack of suitable analytical techniques. Quantitation of lipoprotein classes by lipoprotein electrophoresis was unreliable and ultracentrifugation laborious and unsuited for epidemiological investigations. After the introduction of the precipitation techniques originally described by Burstein (28, 29), HDL and LDL could be isolated accurately and rapidly; selective lipoprotein determinations could now be applied in large-scale epidemiological studies. Since 1975, when Miller and Miller (30) first proposed that HDL could protect against the development of CHD, numerous cross-sectional (31, 32) and prospective (33-36) studies have clearly shown that a low concentration of HDL is a much stronger coronary risk factor than elevated LDL levels. Reduced concentrations of HDL are associated with a number of other risk factors such as cigarette smoking, obesity, physical inactivity and metabolic disorders like diabetes mellitus and chronic renal failure (for reviews see 37, 38).

HDL levels are most frequently measured as HDL cholesterol, but also when the concentrations of other components of the HDL particle are determined, such as apolipoprotein AI, an inverse relationship is found to CHD (39).

Already early in the fifties, Gofman (40) demonstrated that HDL can be subfractionated by ultracentrifugation into two subclasses, HDL₂ and HDL₃. From his laboratory came the first report that the more lipid-rich HDL₂ fraction was a better predictor for cardiovascular risk than total HDL (27), a finding which has later been con-

firmed by others (41, 42).

However strong and consistent, statistical correlations demonstrated in epidemiological studies do not prove a causal relationship between the coronary risk factors and cardiovascular disease. Such knowledge requires experimental studies of the mechanisms for coronary atherogenesis. Also, to correctly interpret the significance of different plasma lipoprotein levels for the progression of coronary atherosclerosis, we must further penetrate the complex metabolism of plasma lipoproteins and extend our knowledge on the interactions between lipoproteins and the vessel wall.

Development of the atherosclerotic lesion

The pathogenesis of atherosclerosis has recently been reviewed by Ross and Glomset (43, 44). The focal lesion is the result of three events: the proliferation of intimal smooth muscle cells, the deposition of intra- and extracellular lipid and the formation of connective tissue matrix by the proliferated smooth muscle cells. These phenomena will result in the various stages of the atherosclerotic process: the *fibromusculoelastic lesion*, the *fibrous plaque* and the calcified *complicated lesion*, which finally precipitates vascular occlusion.

A pre-requisite for these events, according to the *response-to-injury hypothesis* (43, 44), is endothelial injury, disrupting the endothelial barrier and facilitating the ingress of material from plasma into the ar-

terial wall. Potent mitogenic substances, *platelet derived growth-factors* , are then released from platelets which aggregate over areas of endothelial abrasion and stimulate smooth muscle cells to proliferate and migrate into the intimal space. Several of the coronary risk factors can cause endothelial damage. Mechanical injury of the endothelium is believed to take place in arterial hypertension (45). Carbon monoxide and other substances inhaled in cigarette smoke may cause chemical injury of the endothelium (46, 47). Also, chronic hypercholesterolemia with high concentrations of LDL may lead to endothelial break down (48, 49).

In addition to smooth muscle cell proliferation, endothelial damage stimulates invasion of macrophages into the artery wall. These macrophages and the adjacent smooth muscle cells ingest and degrade plasma derived substances that have penetrated the injured endothelial barrier. Since almost all kinds of material except for cholesterol can be degraded by the lysosomal machinery, these events will result in a selective deposition of cholesterol in the vessel wall. The deposition of lipid in the arterial wall is further accelerated by alterations in the intracellular cholesterol metabolism. Under normal conditions a substantial proportion of LDL is taken up via LDL receptors on the cell surface (24); cholesterol entering the cell by this pathway will strongly inhibit the cell's endogenous cholesterol synthesis. This metabolic inhibition will normally prevent an excessive cholesterol accumulation in the cell. However, when the endothelium is damaged, LDL seems to be taken up by the cells predominantly by an alternative mechanism resembling non-specific phagocytosis, the so called *scavenger* pathway (24, 50). Cholesterol entering

via this pathway does not inhibit the cell's own cholesterol synthesis. Consequently, the continued intracellular cholesterol synthesis will contribute to the lipid deposition and to the formation of atherosclerotic plaques.

The third event in the atherosclerotic process, the excess formation of connective tissue matrix is probably due to the proliferating smooth muscle cells, but relatively little is known about the factors that stimulate this process.

It is still incompletely understood how HDL may retard the atherosclerotic process. A hypothetical model for HDL-mediated transport of cholesterol from cells in peripheral tissues to the liver was presented already in 1968 by Glomset (51). Several *beneficial* effects have later been demonstrated experimentally: HDL seems to reverse the harmful effects of LDL on endothelial cells (49) and *in vitro* studies show that HDL enhances cholesterol efflux from erythrocytes (52) and cells in culture (53). Although such studies are complex and difficult to translate to *in vivo* conditions, it has been suggested that HDL might retard the atherosclerotic process by maintaining an optimal cholesterol content in endothelial cell membranes and by promoting cholesterol removal from arterial tissue (54). To find new experimental models for the investigation of the hypothetical anti-atherogenic role of HDL appears to be an urgent subject for future research.

TABLE I Physiochemical properties and biological half-life of plasma lipoproteins.

	Half-life	Ultracentrifugal density (kg/l)	Electrophoretic motility	Particle diameter (nm)
Chylomicrons	minutes	< 0.95	Origin	75-1000
VLDL	hours	0.95-1.006	pre- β	30-80
LDL	9 days	1.006-1.063	β	20
HDL	5 days	1.063-1.125	α	10
HDL ₂ HDL ₃		1.125-1.210		7

TABLE II. Approximative relative composition and major apolipoprotein content of plasma lipoproteins.

	Triglyceride (%)	Free cholesterol (%)	Cholesterol ester (%)	Phospholipid (%)	Protein (%)	Major apoproteins
Chylomicrons	88	3	2	5	2	(apo A) apo B (apo C)
VLDL	55	7	12	18	8	apo B (apo C)
LDL	6	8	42	22	22	apo B
HDL	5	5	12	30	48	apo A apo C (apo E)

Lipoprotein metabolism

Triglycerides and cholesterol, derived from intestinal uptake of dietary fat and from synthesis in the liver, are transported in the circulation as water-soluble lipid-protein complexes, the lipoprotein particles. These particles can be divided into four major lipoprotein classes according to their density (55). The schematic structure of such lipoprotein particles is shown in fig 1 and their composition and physicochemical properties are summarized in tables I and II. Several aspects of the intravascular metabolism of plasma lipoproteins have recently been reviewed (56 - 59). A hypothetical model for the relationship between various lipoproteins and their metabolic pathways is shown in fig 2. Functionally, lipoprotein metabolism can be divided into two systems: the delipidation pathway of VLDL and chylomicrons (the *triglyceride-rich lipoproteins*) to LDL, and the HDL system. These two systems are interrelated and changes occurring in one of the systems will also affect the other.

The principal function of VLDL, chylomicrons and LDL is to transport energy, as triglycerides, and structural components, as cholesterol, from the liver and intestine to peripheral tissues. The end product of this pathway, LDL, is the major carrier of cholesterol in plasma. The role of the HDL system is less well known. An increasing amount of evidence indicates three major functions: first, it serves as a reservoir of apolipoprotein CII, a necessary activator of lipoprotein lipase (LPL); second, it assists the triglyceride-transporting system with a degradation system for the surface components, phospholipid and free cholesterol; third, HDL is

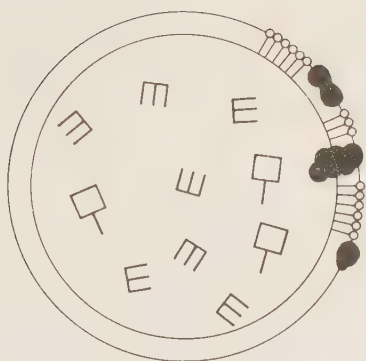


Fig 1. Schematic outline of the structure of plasma lipoproteins. The hydrophobic core components, i.e. triglyceride (E) and cholesteryl ester ($\text{—}\square$) are covered by a surface film consisting of amphipathic components, i.e. apolipoproteins ($\bullet\bullet\bullet$), phospholipid and unesterified cholesterol ($\text{—}\circ$).

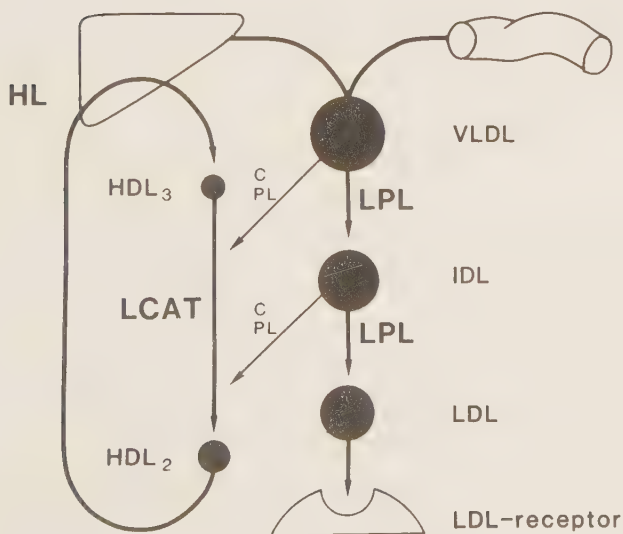


Fig 2. Hypothetical model for the intra-vascular lipoprotein metabolism. PL, phospholipids; C, unesterified cholesterol.

believed to take part in the reversed transport of cholesterol from tissues and other circulating lipoprotein particles to the liver for final degradation and excretion in the bile.

As seen in fig 2, triglyceride-rich lipoproteins are secreted by the liver and the intestine. After transfer of C-apoproteins (including apo CII) from HDL particles, the triglyceride-rich particles can be acted upon by LPL. This enzyme, originating from heart and skeletal muscle as well as adipose tissue, exerts its catalytic action at the luminal surface of the vascular endothelium, where it catalyzes the hydrolysis of the triglyceride core of the triglyceride chylomicrons and VLDL. During the delipidation process, surface components become excessive and are transferred to HDL particles, while the triglyceride-rich particles are transformed into smaller and denser LDL particles. The LDL particles are ultimately taken up by the LDL receptor (24) or the scavenger pathway (50).

HDL originates from disc-shaped precursors secreted by the liver and intestine, so-called *nascent* HDL, which are rapidly transformed into spherical HDL particles by the action of lecithin: cholesterol acyltransferase (LCAT), an enzyme that circulates in plasma associated with the HDL particles. The HDL system receives surface components originating from triglyceride-rich lipoproteins during their degradation. By the action of LCAT, the free cholesterol is esterified and the cholesteryl ester incorporated in the core of the HDL particles. This process is associated with a transformation of smaller HDL particles (HDL_3) to larger, more lipid-rich particles (HDL_2). Part of the cholesteryl

esters formed are transferred from HDL₂ to LDL while the remainder is transported by the HDL₂ particle to the liver for excretion in the bile. Hepatic lipase (HL), another key enzyme in the intravascular lipoprotein metabolism, is believed to be involved in HDL cholesterol elimination by facilitating the uptake of lipoprotein surface components in the liver by virtue of its phospholipase activity. Thereby an HDL₃ particle is regenerated. The ultimate fate of the HDL particles is not known. The half-life of HDL is approximately 5 days. It has been proposed that it is then eliminated by specific HDL receptors in peripheral cells (38).

It is apparent that the intravascular lipoprotein metabolism is largely determined by the activities of the enzymes LPL, HL and LCAT (57). Expressed in a simplified manner, an increase in LPL activity will result in decreasing VLDL and chylomicron levels, whereas HDL levels, especially HDL₂, will rise. In contrast, enhanced HL activities will lead to a reduction in HDL concentrations, especially HDL₂. LCAT, finally, is involved in the regulation of the HDL₂/HDL₃ ratio.

The regulation of the LPL reaction is better known than that of the other enzymes. Three different regulatory mechanisms seem to be involved (for reviews see 57, 59). First, an increased input of triglyceride-rich lipoproteins (VLDL and chylomicrons) will, in normolipidemic subjects, where the triglyceride degradation system is not saturated, lead to an enhanced triglyceride removal and an increased flux along the delipidation pathway, *passive modulation*. Second, LPL activity is strongly affected by certain hormones, *active regulation*. Insulin, for example, is a potent activator, whereas

catecholamines inhibit LPL activity. Third, *cofactors*, especially the proportions of activating and inhibiting apolipoproteins in the substrate, will affect the LPL reaction; apo CII has activating and apo CI and CIII inhibiting properties. The regulation of HL is largely unknown; so far it has been shown to be affected by thyroid hormones (60) and steroid hormones (61 - 63). The LCAT reaction seems to be regulated mainly by the proportions of substrate and products. HDL₃ is the preferred substrate, whereas HDL₂, the product of the LCAT reaction, markedly inhibits LCAT activity (64).

Factors influencing plasma lipoprotein levels

As outlined above, the plasma levels of the lipoprotein classes are determined by a complex interplay between production, intravascular metabolism and elimination of the various lipoprotein particles. A number of factors have empirically been found to affect plasma lipoprotein concentrations; in many cases the mechanisms behind these influences can be recognized. Some examples are given below.

Dietary factors or habits may influence high LDL cholesterol levels (for reviews see 23, 65). When fat intake is reduced and the ratio of polyunsaturated to saturated fatty acids is increased, plasma LDL concentrations decrease. Unfortunately, HDL levels will also drop significantly; especially the anti-atherogenic HDL₂ fraction will be reduced. A high intake of dietary carbohydrates increases VLDL concentrations mainly by accelerated synthesis, and reduces plasma HDL concentrations, reflec-

ting the well-known inverse relationship between VLDL and HDL concentrations (38). In some, but not all studies, high intake of dietary fiber is reported to lead to an elevation of HDL in parallel with a decrease in LDL levels (66). Habitual alcohol consumption markedly raises HDL levels (67, 68), probably by increasing LPL activities (69). Obesity is often associated with low HDL levels (16). During weight-loss, which also increases HDL concentrations significantly, no change in LPL activity is seen, however (70). This effect is probably due to the reduction in body weight rather than caloric restriction (70).

The fact that women have about 25% higher HDL levels than men, mainly owing to differences in the concentration of the HDL₂ subfraction (71), has focused interest on the role of female sex hormones in the regulation of HDL levels (72). Estrogens generally raise HDL levels, while progestogens reduce them. The mechanisms for these changes seem to involve the lipolytic enzymes, since estrogens decreases the activity of HL (61). The rise in HL activities seen during progestin therapy (62), accompanied by decreased HDL levels, also supports current views on the physiological role of the postheparin lipases. Androgens, which reduce HDL levels (63), seem to affect HDL synthesis as well as HL activities (38).

Certain pathophysiological conditions illustrate the impact on plasma lipoprotein levels caused by disturbances in the intravascular lipoprotein metabolism. In hypothyroidism, where elevated LDL levels are a characteristic finding, decreased LDL removal due to impaired LDL receptor function has been demonstrated (75); both LPL and HL activities are low (60). This receptor defect,

as well as the impairment of enzyme activity, reverts in parallel with the lipoprotein changes (60) when patients are put on replacement therapy. In hyperthyroidism, there is a selective increase in HL activities in combination with low HDL concentrations which normalize on treatment (74). The opposite pattern is seen after triiodothyronine administration to healthy volunteers (75). An improvement in the lipoprotein profile, with significant decreases in LDL and increases in HDL levels, is seen in unsatisfactorily controlled diabetes patients after institution of insulin therapy (76). These lipoprotein alterations seem to be mediated via an increase in LPL activity (76).

Apart from diet, alcohol intake and body weight, two further life-style related factors are associated with changes in the plasma lipoprotein profile: smoking and physical activity. In cross-sectional studies, HDL levels are elevated in subjects who exercise regularly (77) and lowered in cigarette smokers (78). The present studies were undertaken to further investigate the relationship between these two coronary risk factors and alterations in plasma lipoproteins; such knowledge might hopefully contribute to a better basis for primary prevention of CHD.

AIMS OF THE STUDY

The aims of the present investigations were

- to study the time-course for changes in lipoprotein concentrations after acute myocardial infarction and the impact of these alterations on the LPL reaction
- to evaluate an intense, short-term program for the modification of the coronary risk profile of patients with advanced CHD
- to study the time-course for the effects of physical training in healthy men on plasma lipoprotein concentrations and composition, especially HDL and its subfractions HDL₂ and HDL₃
- to elucidate the mechanisms of such lipoprotein alterations
- to investigate a possible relationship between two of the major coronary risk factors, smoking and lipoprotein profile
- to describe in detail lipoprotein alterations in smokers and cast light upon possible mechanisms for changes occurring after cessation of smoking

METHODS

Experimental designs

A longitudinal experimental approach was chosen in all the investigations. Although more laborious, this design offers several advantages to a cross-sectional design. Cross-sectional studies rely upon the quantitative determination of the pertinent variables in different populations, which are then compared. Since the biological variation of most variables is quite substantial, large control groups are needed and a large number of subjects must be studied to verify if differences between various groups are statistically significant. Statistical correlations between variables in such studies must be interpreted with great caution. Such correlations do not prove causal relationships.

In longitudinal studies, on the other hand, time-dependent changes in different variables and their time-course can be studied. It is also possible to investigate whether a certain variable is modifiable and to what extent. Furthermore, with a longitudinal approach where each subject represents his own control, the influence of the inter-individual biological variation is eliminated and the effect produced by intervention, for example smoking cessation, is much easier to detect. For these reasons there is also no general need for separate control groups in longitudinal studies. The number of individuals required in longitudinal studies is generally smaller than that needed in cross-sectional studies; the use of paired statistical tests gives a satis-

factory sensitivity and precision in the analysis of data.

The longitudinal design is also well suited to give clues to possible mechanisms behind an observed alteration in a specific variable. Analysis of the time-courses of, for example, the concentration of a lipoprotein class and one of the key enzyme activities, supported by significant correlations between the changes in the two variables, can give strong circumstantial evidence for a causal relationship between these factors. Similarly, the influence of other factors that may modify the variable under investigation (e. g. the influence of diet on the HDL concentrations in smokers) can be more readily studied in longitudinal studies. Two experimental designs can be used in this case: either the other factors are kept constant, to allow the analysis of the changes occurring as a result of intervention as such; or the changes in the other variables is carefully monitored and then analyzed, by statistical methods, in relation to the effect obtained.

In the present studies we included only male subjects, to avoid the marked intra-individual variation in HDL levels in women (71). Both in the studies on exercise and smoking cessation we preferred to allow and carefully monitor spontaneous changes in for example diet and body weight to avoid a too artificial experimental situation. Data were collected at frequent intervals to achieve a more reliable characterization of the events studied. Values of most variables before start of the different studies usually represent the mean of two samples drawn within a week which allows a more reliable definition of baseline levels. When possible, the

samples were stored at -70°C and assayed in one series to minimize methodological variations.

Analytical methods

The analytical procedures are described in detail and referenced in the respective papers. Some techniques of special interest are briefly summarized in this section.

Plasma lipids and lipoproteins

Cholesterol and triglyceride were determined enzymatically. HDL cholesterol was quantitated and characterized in the supernatant obtained after precipitation of LDL and VLDL by MgCl_2 and dextrane sulphate. The coefficient of the HDL cholesterol determination is about 1% within one assay and below 3% between assays. The free and esterified fractions of HDL cholesterol were differentiated by performing the measurement with and without the cholesterol esterase reagent. HDL phospholipids were determined as lipid phosphorus. Apolipoprotein AI was determined in whole plasma by electro immunoassay. This method has a precision corresponding to a coefficient of variation of $< 5\%$. LDL cholesterol was calculated according to the formula of Friedewald.

Separation of plasma lipoproteins into lipoprotein classes and HDL into subfractions with zonal ultracentrifugation

gation was performed by a two-step procedure described in paper V. The lipoprotein classes were quantitated by planimetry of the respective absorbance peaks. The composition of the lipoprotein classes was determined in representative pools of each peak.

Lipolytic enzyme and LCAT activities

Lipoprotein lipase (LPL) and hepatic lipase (HL) activities were determined in post heparin plasma with specific assays (79). The methods show satisfactory precision (C.V. < 5%). LPL activity in adipose tissue and skeletal muscle specimen was determined after elution with heparin (80).

The endogenous cholesterol-esterifying ability was determined according to Stokke and Norum (81). The cholesterol esterification rate was also measured using normal serum as substrate with the technique described by Glomset and Wright (82).

Body compartments

Total body potassium was determined by measurement of ^{40}K in a whole body scintillation counter calibrated with a phantom. Lean body mass (LBM) was determined according to Forbes (83). Body fat weight was calculated by subtracting lean body mass from the body weight.

Diet

Dietary habits were registered by a clinical nutritionist by the *diet history* method described by Burke (84). This technique is frequently used for estimating habitual food intake and has been shown to give at least as adequate results as the laborious and time-consuming *precise weighing* method (85). The Food Composition Tables edited by the Swedish National Food Administration were used to calculate the dietary content of fat, protein and carbohydrates.

LIPOPROTEINS IN ACUTE MYOCARDIAL INFARCTION (Paper I)

Earlier investigations

In the late fifties it was noted that the concentrations of serum lipids and lipoproteins decreased shortly after acute myocardial infarction (AMI) (86 - 88). Similar alterations of the lipoprotein profile with decreased HDL and LDL levels have later been demonstrated by others (116) in other inflammatory states as well (89 - 92).

Present investigation

Patients with AMI provide a dynamic model for lipoprotein alterations *in vivo*. The major purpose of the study was to investigate the impact of the decrease in HDL, and the increase in acute phase proteins, on the LPL reaction.

Twentyone men with myocardial infarction were included in the study. Mean HDL cholesterol and apo-AI levels decreased by about 25% with minimum values after 10 to 14 days. The concentrations of LDL cholesterol were reduced to about the same extent and with a similar time-course. The drop in HDL levels was significantly correlated with variables reflecting the extent of myocardial damage and the degree of inflammatory response, but no such correlations were found for the decrease in LDL cholesterol levels.

To investigate whether the reduction in HDL concentrations after AMI might also affect the LPL reaction we performed a crude cofactor analysis of the patients sera in the following manner: the enzyme activity of a standard amount of purified LPL was assayed with a substrate in which the normal serum ordinarily used as cofactor was replaced by serum from the patients. Thus, the *LPL activating ability* of individual sera could be determined. Sera from day 3 (before any HDL reduction had occurred) and day 10 (when the lowest lipoprotein values were recorded) were compared. Since the incubations were performed with varying amounts of serum added, the activation characteristics could be expressed in terms of $V_{\max}$ and apparent K_M . Values for both these variables differed reproducibly and significantly in sera from days 3 and 10. Another interesting finding was the strong correlations found between plasma concentrations of orosomucoid and the activation characteristics of the sera.

Discussion

The extent and time-course for the decrease in plasma lipoprotein concentrations were consistent with results from earlier studies (89, 90). These changes persisted up to three months after AMI.

The mechanisms behind the reduction of plasma lipoprotein concentrations are not known. The plasma level of lipoproteins is, in general, determined by the rate of production, elimination and intravascular metabolism. However, it is evident that the distribution of lipopro-

tein particles between plasma and the extravascular space will also affect their concentrations in plasma. As a result of an increased permeability of the capillary membranes during an acute inflammatory reaction such as AMI it is possible that small particles like HDL can escape through extravasation into the extravascular space. The transient decrease in plasma albumin levels after surgery is believed to depend partly on such a mechanism (93). The significant correlation between variables reflecting the inflammatory response and the decrease in HDL concentrations is consistent with the hypothesis that the fall in HDL levels is explained by extravasation. However, since there was no change in the proportions of HDL cholesterol to apo-AI after AMI, our data do not support the suggestion that the smaller HDL₃ particles escape preferentially (38).

The larger LDL particle is less likely to disappear from plasma by extravasation to any significant extent. Rather, our data indicate that the reduction of LDL cholesterol levels depends on other mechanisms, since no correlation was found between the changes in HDL and LDL concentrations and since no relation could be demonstrated between the decrease in LDL levels and the degree of inflammatory response. One possibility is an increased removal rate of LDL particles by the LDL receptor pathway or by the scavenger pathway. Chemical modification of LDL such as acetylation has been shown to dramatically increase LDL uptake via the scavenger pathway (60). Another modification of LDL, occurring *in vivo* and resulting from interaction with malondialdehyde released from aggregating platelets, seems to be a prerequisite for cholesteryl ester uptake from LDL by human monocytes and macrophages by the sca-

venger pathway (94). Since platelet aggregation is a characteristic finding in AMI it seems reasonable to suggest that the removal of plasma LDL might be enhanced by an increased uptake via the scavenger mechanisms.

In the present study we tried to test the possibility that the LPL reaction is affected by changes in substrate composition in an *in vivo* system where the concentrations of the apoproteins changed. Apo-CII is a necessary, but normally not rate-limiting, activator of LPL (57, 59), while Apo-CI, apo-CIII and apo-E inhibit LPL activity *in vitro* (95). HDL is regarded as a reservoir for these apolipoproteins *in vivo*. Data from *in vitro* experiments have led to the view that, at least in the rat, the concentrations and proportions of these apoproteins are not rate-limiting for the LPL reaction (96, 97). Although extrapolation from *in vitro* data to *in vivo* conditions should be made with care, the strong relationships between alterations in HDL concentrations and LPL activation raise the possibility that the concentrations and proportions of various apolipoproteins may be of importance for the rate of the LPL reaction under physiological and pathophysiological conditions. Also, the strong relationship between orosomucoid concentrations and the LPL activation indicates that this acid glycoprotein may affect triglyceride metabolism in man, as previously demonstrated in the rat (98).

The physiological implication of these data can be further tested in studies in which the patients' LPL activities are compared with functional measurements of triglyceride turnover. Such studies are in progress.

PHYSICAL EXERCISE AND LIPOPROTEINS IN PATIENTS WITH CORONARY HEART DISEASE (Paper II)

Earlier investigations

Long before the beneficial metabolic effects of physical training (see below) had attracted general attention, exercise therapy had been used for the rehabilitation of patients with recent myocardial infarction (99 - 101). Evaluations of physical conditioning programs generally show that recurrence and mortality rate of myocardial infarction can be reduced by exercise programs (102, 103). In parallel, the patients report good relief of cardiac symptoms, especially angina pectoris (104 - 106). and physical training has gained common acceptance in the treatment of CHD. In the mid-seventies, a number of beneficial effects on lipid and carbohydrate metabolism similar to those found in exercising healthy subjects were demonstrated during cardiac rehabilitation programs (107 - 110). Rather low-intensive, infrequent out-patient training over a long period of time has so far been practiced almost invariably (111 - 114). However, the drop-out rate, especially of severely disabled patients, is often high in such programs (113 - 114).

Present investigation

Anxiety is often the limiting factor in the rehabilitation of coronary patients (115), a fact that may largely explain the low adherence in out-patient training

programs of patients with severe angina pectoris (113, 114). The purpose of this investigation was to evaluate a rehabilitation program with better prospects of patient adherence. We chose a rather intensive and short-term, in-hospital exercise program and studied its psychological, physiological and metabolic effects in a group of patients with severe, stable angina pectoris. All were potential candidates for coronary by-pass surgery but the majority of the patients was negative to operation.

The patients spent nine weeks in a Rehabilitation Center. The exercise consisted of two strictly supervised 30 min sessions per day, and was performed as interval training on an ergometer bicycle. The work loads were gradually increased during the nine weeks program, so that the intensity was always as high as tolerated.

The physical performance, which initially was markedly reduced, improved during the training program and remained significantly better than before training six months after discharge. As a matter of fact, most of the patients have subsequently been followed in our out-patient clinic and still report, two years after the training period, persistent improvement.

In parallel with an increased pain threshold for angina pectoris nearly all patients reported reduction of fear and tension and higher self-esteem. About one third of the patients already had some kind of sick pension before admission to the Rehabilitation Center; of the remaining patients about half managed to go back to work.

Plasma insulin levels decreased, while glucose toleran-

ce improved significantly after the training program; this combination indicates an increased peripheral insulin sensitivity. Plasma LDL levels decreased slightly while HDL levels remained unchanged.

Discussion

The improvement in physical performance achieved in this intensive, short-term rehabilitation program compares favorably with that obtained in long-term out-patient exercise programs (112, 113, 116). The results on psychosocial factors and return to work are also consistent with the experience of others using different training models (117, 118).

Impaired glucose tolerance especially in combination with high levels of plasma insulin increases CHD risk considerably (119, 120). The present exercise program led to a significant improvement of glucose tolerance and a reduction of plasma insulin concentrations, which must be considered beneficial. Improved oral glucose tolerance is occasionally seen after physical training (109), whereas reduction in plasma insulin levels seems to occur more frequently (for review see 121). The basic mechanisms for this phenomenon are not fully clarified but it seems clear that both a decreased secretion rate and an enhanced peripheral clearance of insulin are of importance (121).

The initial plasma lipoprotein concentrations, with low HDL and high LDL levels are well in line with previous epidemiological studies of CHD patients (31).

The results of training on the plasma lipoprotein profile are still difficult to interpret (see following section). The results obtained in investigation of patients with CHD are as complex as those reported in studies of normal subjects. For example, we could not demonstrate any increase in HDL levels, in contrast to reports from other CHD rehabilitation programs (107, 108, 110). These diverging results may be due to the different designs of the training program (see below). A decrease in LDL levels, on the other hand, seems to be a more consistent finding in most exercise studies.

In conclusion, the present model with daily, in-hospital training of comparatively high intensity and short duration gives results comparable to those obtained in traditional out-patient exercise programs with regard to physiological, psychological and metabolic effects. It may therefore be considered as a complement to pharmacological therapy and an alternative to coronary surgery in selected cases.

LIPOPROTEINS AND PHYSICAL EXERCISE IN HEALTHY MEN (Paper III)

Earlier investigations

As pointed out in the background section, physical inactivity is regarded as a risk factor for CHD (122). In large retrospective as well as prospective epidemiological studies, subjects with high physical activity have a lower incidence of CHD (10, 11, 123, 124). Although no direct proof exists that exercise prevents or retards coronary atherosclerosis, there is increasing circumstantial evidence supporting this view. Thus, a number of beneficial cardiovascular and metabolic effects of physical training have been identified (125). Although the basic mechanisms remain speculative, the anti-atherogenic effects of exercise may partly be mediated via effects on plasma lipoproteins.

Already about twenty years ago it was reported that participants in a long-distance cross-country ski race had low levels of VLDL and high levels of HDL (126). Ten years later, when HDL had been indicated as a negative risk factor for CHD (30), this finding was rediscovered through the work of Wood *et al* (127). During subsequent years numerous comparative studies have confirmed the high HDL levels in physically active men (for review see 77). The rapidly increasing information in this area has recently been collected by Hietanen (128).

Present investigation

Although it was obvious from cross-sectional studies that physically fit subjects had higher HDL and lower LDL and triglyceride levels in plasma than sedentary persons (77), it remained to be demonstrated that corresponding changes in the lipoprotein profile would occur when inactive subjects started to exercise. It took several years before experiences from longitudinal training studies started to accumulate and, when the present investigation was planned, only scarce and conflicting data from such studies were available (129-132). The discrepancies in the literature were believed to depend on differences in the intensity, frequency and duration of exertion and on variable effects of the training program on the participants' body weight and energy intake (77). The mechanisms behind the lipoprotein alterations had not been extensively studied.

Our purpose was to investigate the effects of strictly supervised, longitudinal aerobic training of varied intensity, frequency and duration on the plasma concentrations of the various lipoprotein classes and their composition. Changes in body weight and composition, diet, alcohol intake and smoking habits were carefully registered. To elucidate possible mechanisms behind the lipoprotein alterations the activities of the key enzymes in intravascular lipoprotein metabolism, LPL, HL and LCAT were measured and related to the plasma concentrations of the lipoproteins.

The subjects trained for six to twelve weeks on ergometer bicycles three to five times weekly for about one hour at 70 - 90% of estimated maximal oxygen uptake.

A physiotherapist and a physician were present at all training sessions, continuously recording heart rates and workloads. It was thus possible to estimate the intensity of exertion and individual energy expenditure during the training program.

All blood samples and tissue biopsies were obtained 2-3 days after the latest training session. Pilot experiments in twelve of the subjects had demonstrated that and acute effects of training had vanished 48 h after a training session. Blood samples were collected at frequent intervals, allowing a reliable characterization of the entire time-course of lipoprotein changes.

Plasma HDL concentrations showed a moderate, transient increase, which was most pronounced in subjects training at a lower intensity. All HDL components, except for HDL triglyceride, increased in parallel leading to moderate changes of HDL lipid composition. In a subsample of the subjects a preferential increase in the HDL₂ subfraction could be demonstrated. LPL activity in adipose tissue increased markedly whereas HL activities decreased moderately. The rise in HDL was related to changes in percent body fat, dietary fat intake and increase in LPL activities. The increase in HDL levels was inversely correlated with the intensity of exertion, but we could find no relationship to the frequency of training sessions or the total amount of training performed.

Discussion

The increase in maximal oxygen uptake ($\text{VO}_{2\text{max}}$) was com-

paratively pronounced (133 - 135). This seems to be of little importance for the changes in HDL concentrations since there is generally no relationship between VO_2 max and HDL levels (132, 134 - 136). There are, as a matter of fact, several exercise studies showing a marked improvement of the maximal aerobic power but no rise in plasma HDL concentrations (133, 137, 138). However, many studies show a significant rise in plasma HDL levels (132, 134, 136) with training. The increase in HDL, measured either as HDL cholesterol or apolipoprotein AI, is usually about 10% (132, 134, 136), which is in agreement with our findings. Also, the initial time-course for the increase in HDL is similar to that reported previously (135). However, the elevation of HDL levels was most prominent during the initial adaptation to exercise and tended to dampen out with prolonged training. The reason for this decline in HDL, which has not been evident in other studies, is not clear. The phenomenon, however, is important to note since it could explain the lack of HDL elevation in studies where HDL concentrations are measured only before and after a longer training period (138).

The contradictory results with respect to lipoprotein alterations in different training studies are subject to intense debate (77). Among other factors, the rise in HDL levels during physical conditioning has been ascribed to weight loss (132, 139), which is known to influence the lipoprotein profile (70). Specifically, there seems to be a significant relationship between the body fat weight and plasma HDL levels (70), and weight reduction in overweight subjects generally leads to increases in HDL concentrations (70, 131, 140, 141). However, the variation in body weight in this and in most other stu-

dies on physical exercise is small, and the relation between changes in body weight and HDL levels is generally weak or absent when analyzed statistically (132, 134, 136). Measurements of body fat therefore seem necessary to detect correlations between alterations in body compartments and HDL concentrations. The presence of such a relationship in this study indicates that a loss of body fat, although comparatively small, may contribute to the rise in HDL concentrations during exercise programs.

Alterations in diet, especially changes in food composition and fat intake markedly affect HDL levels. A reduction in the total fat intake and an increase in the ratio of polyunsaturated to saturated fatty acids. drastically reduced HDL concentrations in a group of normolipidemic subjects (142). Similar effects of a low fat diet have been reported by others (143). The opposite phenomenon has been noted during the treatment of hyperlipidemic patients with fat-rich diets (144). In this study we found a significant correlation between the increase in fat ingestion and the elevation of HDL cholesterol concentrations. This indicates that changes in diet also contribute to the elevation of HDL during training. As pointed out earlier, an increased input of chylomicron triglyceride, in normolipidemic subjects, leads to an enhanced triglyceride removal via *passive modulation* (59) and gives rise to an increased turnover of chylomicrons, a process associated with an elevation of HDL levels (57).

Differences in the design of the training program might contribute to the variability of the effects on the lipoprotein profile. Among the factors most frequently

discussed are the duration of the training program, the frequency of the sessions, the total amount of training performed, and the training intensity.

When comparing the results of different longitudinal exercise studies, the time-course of HDL changes and the duration of the training period must be taken into account. Usually it takes 2-4 weeks of exertion before HDL changes are observed (131, 135). These data conform with our findings but contrast to another recent study where changes in the lipoprotein profile did not occur before nine months of training (141).

The importance of training frequency remains to be established, since there are, so far, no systematic studies in this area. In the present study, however, no difference in HDL response was observed between subjects exercising three and five times per week.

In both cross-sectional and longitudinal studies it has been demonstrated that the increase in HDL levels is related to the amount of exercise (141, 145). However, this relationship differs considerably between different studies. In the longitudinal study by Williams et al (141) the threshold for lipoprotein changes was running about 9 miles (15 km) per week, whereas in the cross-sectional study by Lehtonen *et al* (145) higher HDL cholesterol values were observed only when the subjects ran more than 70 km per week. In the present study we could not detect any relationship between HDL concentrations and total energy expenditure during the program.

The intensity of exertion seems to be of even greater importance than the duration, frequency and amount of

exercise when the effects of training on plasma HDL levels are evaluated. In physical work of high intensity muscle metabolism is mostly anaerobic. Exhaustive exercise can therefore only be sustained for short periods of time. During more long-lasting, dynamic training, as in the present study, work intensities must be kept at a lower level and muscle metabolism will be predominantly aerobic. Only athletes performing aerobic sports, such as endurance-runners, soccer and tennis players have elevated plasma HDL concentrations, whereas the lipoprotein profile of sprinters and ice-hockey players, mostly engaged in anaerobic training, does not differ from that of controls (146 - 149). The training of ice-hockey players aims at maintaining pulse rates above 180/min while soccer players train at pulse levels of about 140/min (148). Consequently, ice-hockey players spend about three times more energy per minute during their highly intensive training (150). The phenomenon that changes in HDL levels seem inversely related to the intensity of the training program has also been noted in a longitudinal study (151) where reducing training hours and increasing exercise intensity lowered HDL cholesterol levels. In the present study, HDL concentrations increased most prominently in the subjects who trained at the lowest intensity. Furthermore, there was a significant inverse correlation between the intensity of the training program and changes in HDL cholesterol levels. These findings support the view that comparatively low intensive training is especially effective in raising plasma HDL. On the other hand, when training intensity is too low no changes in HDL concentrations occur (135, 137).

The mechanisms behind the inverse relationship between

training intensity and rise in HDL concentrations are complex. However, it is reasonable to assume that this phenomenon can, at least partly, be explained by differences in substrate fluxes in plasma and in differences in substrate utilization in the muscle during work of high and low intensity (152 - 154). In this context, the levels of circulating free fatty acids seem to be of special interest. For instance, endurance runners (148) have significantly higher fatty acid levels than ice-hockey players (145). High concentrations of fatty acids will enhance VLDL synthesis, which will in turn increase the flux along the delipidation path of triglyceride-rich lipoproteins and promote an increase in HDL levels (57).

The mechanisms whereby exercise raises HDL levels are not completely understood, but evidence is accumulating that the rise in HDL concentrations is partly mediated via changes in the activities of the lipolytic enzymes LPL and HL occurring during training. LPL catalyzes the degradation of triglyceride-rich lipoproteins, a process leading to increases in HDL levels; consequently an increase in LPL activities will result in an elevation of plasma HDL concentrations. An increase in HL activities will, on the other hand, lead to a reduction in plasma HDL levels, since HL is believed to be involved in the degradation of HDL particles in the liver (155). In the rat, exercise increases LPL activities in heart and skeletal muscle (156, 157). In man, cross-sectional studies demonstrate two to three fold higher LPL activities in adipose tissue and skeletal muscle from long-distance runners than from sedentary controls, whereas sprinters do not differ from controls (177). Furthermore, LPL activity in adipose tissue is correlated with the amount of ki-

lometers run weekly (146). LPL activity in post heparin plasma is not elevated in physically active subjects (158), but HL activity is significantly lower than in sedentary controls (158) and inversely related to physical fitness (159). The effect of a single exposure of strenuous exercise on LPL activities has been studied (160, 161), showing increases in both adipose tissue (160) and skeletal muscle LPL activities (162), when the exposure time exceeds one hour (cf 161).

Extending earlier cross-sectional studies the present investigation demonstrates that both LPL and HL activities change in subjects who start regular training. The increase in adipose tissue LPL and the decrease in HL activities conform with the results of an earlier longitudinal study (136). The positive correlations between LPL activities and HDL levels as well as the negative correlations between the latter variable and HL activities are consistent with the observation of others (136, 159) and support present views on the physiological roles of the post heparin lipases (57).

The increases in HDL total cholesterol and apolipoprotein AI was accompanied by differential changes in the other HDL components, resulting in significant alterations in the lipid composition of the HDL particles. Such changes could be due to either qualitative alterations in HDL composition or to quantitative changes in the proportions of the lipid-rich HDL₂ and the denser HDL₃ subfractions. As discussed in more detail in paper V, both these mechanisms seem operative in the present experiment: the increase occurred mainly in the HDL₂ subfraction, but there were also slight changes in HDL composition with a relative increase in HDL core

components. These results are consistent with earlier cross-sectional studies showing that HDL₂, but not HDL₃ levels are elevated in runners (159, 163). In accordance with our findings, other groups have also reported a selective increase in HDL₂ during physical conditioning programs (58, 164).

In conclusion, the present investigation shows that physical conditioning can raise HDL levels in sedentary men. The rise in HDL concentrations is most prominent during the initial adaptation to exercise and seems more pronounced in subjects training at comparatively low intensity. The increase in HDL is mainly confined to the anti-atherogenic HDL₂ subfraction. Alterations in body composition and diet appear to contribute to the elevation of HDL induced by the physical exertion as such. The effect of training on HDL seems partly to be mediated via changes in the activities of the lipolytic enzymes, LPL and HL.

Although HDL can be increased in inactive subjects through exercise programs, it remains to be established, in prospective trials, if this effect will also reduce the incidence of cardiovascular disease. Not until such data are available should the positive effects on HDL concentrations be used as an argument for large-scale fitness campaigns.

Earlier investigations

Since Hammond and Horn in 1954 showed that the excessive mortality among cigarette smokers is largely due to coronary heart disease (165) the relation between smoking and coronary atherosclerosis has been studied extensively. Classical, large epidemiological studies have demonstrated that heavy smokers run a five to ten fold greater risk than non-smokers to die from CHD (166, 167). The cardiovascular risk increases progressively with the number of cigarettes smoked (168) and is related to carboxy hemoglobin levels (169). Autopsy studies demonstrate that coronary atherosclerosis is more severe in heavy smokers than in non-smokers (170). The cardiovascular risk is substantially and rapidly reduced after cessation of smoking (171).

Despite almost thirty years of intensive research the basal mechanisms by which smoking accelerates the atherosclerotic process have not been identified. It seems reasonable to assume that the etiology is multifactorial since cigarette smoke contains several thousand chemical compounds. Many substances in tobacco smoke are pharmacologically active, mutagenic, antigenic and toxic. Cigarette smoke is believed to trigger a number of effects that might accelerate the atherosclerotic process such as platelet aggregation and endothelial damage (for review see 172). The properties of carbon monoxide and nicotine, the two components of cigarette smoke most commonly discussed in relation to the vasculotoxic

effects, have recently been reviewed in detail (173, 174). Their relation to atherogenesis will be summarized briefly.

Carbon monoxide, due to its extremely high affinity to hemoglobin, causes marked hypoxia in tissues. Animal experiments and in vitro studies indicate that hypoxia induces damage to the vascular endothelium, increases the permeability of the endothelial barrier to lipids, stimulates proliferation of smooth muscle cells and enhances LDL uptake in smooth muscle cells (175 - 177). However, carbon monoxide has never convincingly been shown to accelerate the development of atherosclerosis in the intact animal (174). Nicotine, via catecholamine release, exerts numerous hemodynamic effects, but it has not been proven atherogenic. Animal experiments indicate that both nicotine and carbon monoxide influence plasma lipids, but their effects on plasma lipoproteins are conflicting (174). There is no doubt that nicotine promotes lipolysis and increases serum free fatty acid levels, but at least in animal experiments it seems to have little or no effect on plasma lipoprotein levels (173). Carbon monoxide has been shown to influence hepatic lipid synthesis and to raise serum cholesterol levels (173) in animal experiments. However, the increased incidence of coronary atherosclerosis in smokers does not seem to be fully explained by the actions of these two compounds.

Alterations in the lipoprotein profile of smokers have been known since 1955, when Gofman *et al* (178) demonstrated that smokers had slightly higher levels of atherogenic lipoproteins than non-smokers. Cross-sectional studies during the sixties and early seventies showed

slightly to moderately elevated serum cholesterol levels in cigarette smokers (for review see 179). Bronte-Stewart was the first to observe in 1961 (180) that smokers, in addition to elevated LDL concentrations, had consistently lower HDL levels. This finding was confirmed in the late seventies when the inverse relationship between HDL and CHD had been discovered (30) and was corroborated in several large epidemiological investigations (for reviews see 78, 179).

Present investigations

The lack of longitudinal studies on the effects of smoking cessation prompted us to follow plasma lipoprotein levels in two groups of heavy smokers after they stopped smoking.

Our purpose was to investigate whether the reduced levels of HDL in smokers could be improved by intervention. We were also interested to study whether the low HDL concentrations were due to smoking as such or could be attributed to other factors in the smokers life-style. Complete abstinence from smoking was assured by frequent measurements of COHb levels. We had a drop-out rate of about 50%, which must be accepted in smoking withdrawal programs. Factors known to affect plasma lipoprotein concentrations, such as changes in body weight, diet, alcohol intake and physical activity were carefully monitored and related to changes in the lipoprotein profile. Alterations in the composition of the various lipoprotein classes, including HDL₂ and HDL₃ subfractions, were studied. In an attempt to elucidate possib-

le mechanisms for the lipoprotein alterations, the activities of the key enzymes in lipoprotein metabolism, LPL, HL and LCAT, were determined.

The detailed result of these studies are given in papers IV and V. In summary, moderate increases in caloric intake, especially fat, were noted after smoking cessation, but the weight gain was insignificant. HDL concentrations rose rapidly and markedly while LDL levels decreased slower and to a lesser extent. The increase in HDL levels occurred mainly in the HDL₂ subfraction. The activities of LPL demonstrated a rapid, transient increase whereas HL activities remained constant. There was also a rise in LCAT activities after smoking cessation. Correlation analyses are consistent with the interpretation that the increase in HDL levels is partly due to the increased fat intake and partly mediated via the increases in the activities of LPL and LCAT.

Discussion

Although the differences in HDL levels between smokers and non-smokers in cross-sectional studies (78) can amount to 10-15%, we were surprised by the rapid and pronounced increase in HDL concentrations occurring after smoking cessation. This finding has later been confirmed (181). The rise in HDL is in the same order of magnitude as the difference between men and women (71). If we assume that the low CHD incidence in women is due to their higher plasma HDL levels this would imply that the increased rate of cardiovascular disease in smokers could to a great extent, be explained by the diffe-

rences in HDL concentrations between smokers and non-smokers. HDL is generally considered a comparatively constant variable, and only after long-term alcohol consumption (69) are changes of this order of magnitude seen. In contrast to the finding during exercise, HDL levels remained elevated after smoking cessation and showed no tendency to drop again. When some of the subjects resumed smoking, however, their plasma HDL levels rapidly fell again. A decrease in LDL levels, although less prominent, contributed to the improvement in the lipoprotein profile after smoking cessation. The increase in HDL was mainly confined to the anti-atherogenic HDL₂ fraction, which was low or virtually absent before stopping smoking.

The mechanisms for the changes in LDL concentrations after smoking cessation remain speculative. The rise in HDL levels, however, seems related to an enhanced degradation of triglyceride-rich lipoproteins since a highly significant correlation was found to the increase in fat ingestion (i. e., input of chylomicron triglyceride) and to the increase in LPL activity; both these factors lead to an increased flux along the delipidation pathway of triglyceride-rich particles, a process associated with an elevation in plasma HDL concentrations. By extrapolation from the correlation between these variables it can be estimated that about 50% of the HDL increase is related to increased fat ingestion and about 25% due to increased LPL activity.

Smoking cessation seems, due to the pronounced changes in HDL concentrations, a model well suited for the study of factors influencing intravascular lipoprotein metabolism. For example, the effects of diet and LPL on HDL

levels could be distinguished by keeping the diet as constant as possible after smoking withdrawal. It is also unclear which components in cigarette smoke are active in affecting HDL levels. This could be clarified by different experimental approaches, for instance a smoking withdrawal program with and without the use of nicotine chewing-gum. Such studies are in progress.

CONCLUDING REMARKS

Coronary heart disease represents a large medical and economic burden to most developed countries. During the past fifteen years, death from CHD has decreased considerably owing to better and more effective therapy of patients with acute myocardial infarction and other cardiovascular disorders. It is evident, however, that a reduction in the incidence of CHD can be reached only by primary prevention of the disease.

The importance of the major coronary risk factors for CHD morbidity is generally accepted. It is, however, still debated if modification of a coronary risk factor will lower the incidence of cardiovascular disease. So far, it has been convincingly proven that cessation of cigarette smoking and adequate treatment of hypertension decrease coronary risk and CHD mortality (171, 182). The beneficial effects of reducing normal or slightly elevated serum cholesterol concentrations are, however, still open to doubt. Substantial reductions of serum cholesterol levels can be achieved in primary intervention studies by diet alone (183) or by drugs (184). Although CHD mortality is reduced, the results of these studies are disappointing. Overall-mortality is not affected by dietary intervention (183) and even increases when clofibrate, a lipid lowering drug, is used (184).

In order to improve future intervention trials, particularly such devoted to the modification of the plasma lipoprotein profile, it seems essential to further elucidate the mechanisms that influence the progression of coronary atherosclerosis. For example, the impact of

HDL₂ and HDL₃ subfractions and that of various HDL components, e.g. the apolipoproteins, on the atherosclerotic process must be studied in more detail.

Since the effects of intervention on plasma lipoproteins are extremely complex, it is also desirable that intervention programs are carefully tested in smaller studies where the metabolic and biochemical effects of intervention are documented in detail. Information from such pilot experiments may hopefully improve the design and focus of future large-scale intervention studies. The rapid development of diagnostic techniques to follow the progression or regression of atherosclerosis will probably greatly facilitate this approach. Modern angiographic methods (185), for instance, makes it possible to evaluate the effects of intervention long before the patients reach the *end-points* traditionally used to evaluate intervention programs.

Although present knowledge on lipoproteins and atherogenesis is incomplete, it seems reasonable and is generally agreed that risk factor elimination is of importance for individuals with unfavorable coronary risk profiles (5). However, it is still premature to use *elevation of HDL* as an argument for public health campaigns.

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Alterations in plasma proteins and lipoproteins in acute myocardial infarction: effects on activation of lipoprotein lipase

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Stubbe, I., Gustafson, A. & Nilsson-Ehle, P. Alterations in plasma proteins and lipoproteins in acute myocardial infarction: effects on activation of lipoprotein lipase. *Scand. J. clin. Lab. Invest.* 42, 437-444, 1982.

Plasma lipoprotein concentrations were followed in 21 men with acute myocardial infarction. HDL and LDL cholesterol concentrations showed similar time-courses with average maximal decreases of about 20%, 10-14 days after onset of symptoms. The decrease in HDL levels (measured as HDL cholesterol and apolipoprotein AI) was significantly correlated to the inflammatory response, as reflected by plasma orosomucoid concentrations, and to the extent of myocardial injury, as mirrored by serum activities of lactate dehydrogenase. In samples drawn 10 days after myocardial infarction we found marked changes in the ability of the patients' sera to enhance the activity of purified lipoprotein lipase. The maximal activating ability (at saturating serum concentrations) increased by about 30%; however, at suboptimal serum concentrations, the activating ability of the patients' sera declined (50% higher serum concentrations were required to reach half maximal reaction rate). The altered activation characteristics were correlated to the changes in HDL concentrations. By affecting the activity of lipoprotein lipase and thereby the rate of intravascular lipoprotein metabolism, this phenomenon may contribute to the lipoprotein alterations seen after myocardial infarction.

Key-words: acute myocardial infarction; apolipoprotein AI; high density lipoprotein; lipoprotein lipase; orosomucoid; plasma lipoproteins; serum lactate dehydrogenase

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A transient decrease in plasma lipoprotein concentrations is a characteristic finding in acute myocardial infarction (AMI) as well as in other inflammatory states [1, 8, 10]. For example, high density lipoproteins (HDL) show

a rapid decrease, reaching a minimum about one week after infarction with restoration to normal values after about 3 weeks [8], a time-course similar to the changes seen in plasma albumin concentrations.

The mechanisms responsible for these changes are still obscure. Decreased synthesis and

secretion of lipoproteins from the liver or intestine or accelerated elimination from the blood stream by extravasation have been suggested [8]. In addition, alterations in the intravascular metabolism of lipoproteins—especially the lipoprotein lipase (LPL) reaction, which is associated with the formation of HDL [14]—may be of importance.

The aim of the present investigation was to study the relationship between plasma lipoprotein alterations and variables reflecting the extent of myocardial injury, and the degree of inflammatory reaction after AMI. Specifically, we wanted to investigate whether changes in the lipoprotein profile after AMI would also affect the LPL reaction, thereby possibly constituting an accessory mechanism to explain the course of lipoprotein changes after AMI.

MATERIAL AND METHODS

Patients. We studied 21 male patients with AMI, consecutively admitted to the Coronary Care Unit of the University Hospital, Lund, Sweden. Their mean age was 56 years (range 41–58). None of the patients was grossly overweight; mean Broca's index was 1.05* (range 0.81–1.21).

The diagnosis of AMI was based on at least two of the following criteria: typical chest pain lasting for at least 30 min within 12 h before admission to the hospital, typical ECG changes according to the Minnesota code [18] and a characteristic increase in the serum ASAT activities.

A minority of the patients had a history of earlier disease. Two patients took beta₂-stimulants for bronchial asthma; one also used corticosteroid inhalations. Two were medicating with allopurinol for gout, one had been surgically treated for carcinoma *in situ* of the urinary bladder and two were on dietary treatment, one for mild hypercholesterolaemia, the other for diabetes mellitus. The latter patient had to be put on insulin during the course of AMI. There were three patients with arterial hypertension, five with angina pectoris and three with earlier MI, in all instances more than one year prior to the present admission. Of these a total of five were taking beta-blockers and one digoxin.

The ECG revealed transmural myocardial damage in all cases but four, who had ECG changes indicating subendocardial infarction. The MI was located inferiorly in five patients and anteriorly in the others. The changes in serum ASAT and LD activity concentrations were those expected in AMI as were the LD isoenzyme patterns at day 3–5.

During the observation period two patients with early ventricular fibrillation were successfully defibrillated and given lidocain infusions. One patient with ventricular arrhythmia was treated with disopyramid. Various supra-ventricular arrhythmias were treated with beta-blockers in seven cases, while five patients were treated with digoxin and diuretics, mainly furosemide for mild cardiac decompensation. A total of twelve patients were put on anti-coagulant therapy.

The LPL activating ability of the patients' sera were studied in a subgroup of six subjects. None of them received drugs (insulin, beta-blockers) that might affect lipid metabolism.

Blood sampling. The first blood sample was drawn immediately after arrival to the Coronary Care Unit within 12 h after onset of symptoms. Samples obtained on days 3, 5, 7, 10 and 14, and after 1 and 3 months were drawn in the fasting state, the last two on visits to the outpatient clinic. Plasma samples for determination of proteins and lipoproteins were collected using EDTA as anticoagulant, whereas serum was used for the other analyses.

Laboratory analyses. The concentration of plasma orosomucoid, ceruloplasmin and apolipoprotein AI were measured by electro-immunoassay and expressed relative to the value of a serum pool obtained from 40 healthy blood donors [11]. LD and ASAT activities in serum were measured by kinetic techniques using reagents from AB Kabi, Stockholm, Sweden. Plasma lipoprotein electrophoresis was performed on 0.8% agarose gel [9].

Plasma cholesterol [17] was measured enzymatically with reagents from Boehringer Mannheim, West Germany. The fraction of plasma cholesterol associated with HDL (HDL cholesterol) was quantified by precipitation of VLDL and LDL by MgCl₂ and dextrane sulphate [2] with subsequent determination of cholesterol in the clear supernatant. LDL

*Broca's index = $\frac{\text{body weight (kg)}}{\text{height (cm)} - 100}$; reference interval 0.8–1.2.

cholesterol was estimated from the equation, LDL cholesterol (mmol/l) = plasma cholesterol (mmol/l) — [plasma triglycerides (mmol/l) $\times$ 0.45 — HDL cholesterol (mmol/l)] [6].

Plasma triglycerides were determined enzymatically [20]. In these patients, who all had plasma triglyceride levels below 5.0 mmol/l, and where the absence of chylomicrons was verified by electrophoresis, plasma triglyceride levels can be expected to reflect essentially plasma VLDL concentrations.

LPL activating ability. Postheparin was obtained from healthy volunteers 15 min after the injection of 50 U heparin (Vitrum, Stockholm, Sweden) per kg body weight [15] and collected in EDTA-containing Vacutainer tubes. LPL was separated by repeated affinity chromatography on Heparin-Sepharose [3]. The enzyme, obtained by pooling the peak of lipase activity eluting between 1.2 and 1.5 mmol/l NaCl, was free from hepatic lipase activity, as evidenced by virtually total inhibition by 1 mmol/l NaCl in the assay system and by its serum dependence (about 10% of optimal activity was recorded in the absence of serum). The enzyme was stored in portions at -70°C .

LPL activity was assayed as described earlier [15], using as substrate a tri[^3H]oleoyl-glycerol emulsion stabilized by lecithin. For the study of LPL activation, the substrate was modified by substituting a 4% (w/v) solution of bovine serum albumin (crystalline quality Sigma, St Louis, Mo.) for the serum ordinarily used in the assay.

The ability of the patients' sera to enhance LPL activity was determined by assaying the activity of purified LPL in the absence of serum and in a series of incubations containing increasing amounts of the patients' serum. Enzyme (50 μl diluted 1 + 2 with distilled water, NaCl concentration 0.45 mmol/l) was mixed with 5–50 μl of serum (diluted 1 + 15 in 0.15 mmol/l NaCl); the volume was adjusted to 100 μl by the addition of 4% (w/v) bovine serum albumin (diluted 1 + 15 in 0.15 mmol/l NaCl). The reactions were then started by the addition of 100 μl substrate solution. The final concentrations in the incubations were triacylglycerol, 1.25 mmol/l; lecithin, 0.08 mmol/l; bovine serum albumin, 0.27% (w/v) in 0.1 mmol/l Tris-HCl buffer (ph 8.0) containing

0.125 mmol/l NaCl. The serum concentration ranged from 0.16 to 1.6% (w/v); the variations in albumin concentration resulting from the varying serum additions were compensated for so that the final albumin concentration, including the albumin added with the serum, was always 0.33%. Incubations were carried out for 15 min at 37°C .

The enzyme activities in each series ranged from 0.01 to 0.13 $\mu\text{kat/l}$. When the reciprocal of enzyme activity was plotted against the reciprocal of serum concentration, data yielded a straight line which allowed the calculation of maximal reaction rate (V_{max}) and apparent K_{M} (K_{Mapp}), the latter entity designating the serum concentration necessary to provide half V_{max} .

The LPL activating ability was studied in a subsample of six patients, in which we compared samples drawn on day 3 (when lipoprotein concentrations were still essentially unchanged) and day 10 (when the lowest concentrations of lipoproteins were recorded).

Statistical analyses. The significance of changes in different variables during the course of AMI was evaluated using Wilcoxon's rank order test for paired observations. Correlations between different variables were analysed by linear regression analyses.

RESULTS

Acute phase proteins. Plasma orosomucoid concentrations reached an average maximal increase of 70% ($P < 0.01$) on day 7, while ceruloplasmin concentrations had a maximum with a mean increase of 43% ($P < 0.01$) on day 10 (Fig. 1). Both variables returned to initial levels after three months.

The levels of the acute phase proteins were correlated to the S-ASAT and S-LD activities, indicating a correlation between the extent of myocardial injury and the inflammatory reaction. Maximal S-LD activities showed the best correlation to maximal plasma orosomucoid concentrations ($r = 0.70$, $P < 0.001$).

Plasma lipoproteins and apolipoproteins. The HDL cholesterol concentrations decreased significantly during the observation period, with a mean maximal reduction of 27%

($P < 0.01$) on day 14 (Fig. 1). The time-course for the reduction of plasma apo-AI levels was essentially similar to that of HDL cholesterol with its minimum on day 10, 22% ($P < 0.01$) below the initial value (Fig. 1). The concentrations of HDL cholesterol and apo-AI were strongly correlated ($r = 0.82$, $P < 0.001$).

The decrease in HDL cholesterol was significantly correlated to the maximal activities of S-LD ($r = 0.67$, $P < 0.01$), S-ASAT ($r = 0.55$, $P < 0.05$) and the peak concentrations of plasma orosomucoid ($r = 0.46$, $P < 0.05$). Similarly, changes in apo-AI were also correlated to variables reflecting the extent of myocardial injury and the degree of inflammatory reaction.

The LDL cholesterol concentrations (Fig. 1) demonstrated a time-course similar to that of HDL cholesterol, with a maximal mean reduction of 22% ($P < 0.01$) on day 10. However, we could find no relationship between the individual decreases in LDL cholesterol and HDL cholesterol or apo-AI levels, and in contrast to the changes in HDL concentrations, the decrease in LDL was not positively correlated

to variables reflecting the extent of myocardial injury or degree of inflammatory reaction.

The changes in plasma triglyceride concentrations occurring during the initial part of the observation period (about 30% on day 7) were not statistically significant.

LPL activating ability. The alterations in plasma protein and lipoprotein profile occurring between day 3 and day 10 were accompanied by significant changes in the pattern whereby serum enhanced the activity of purified LPL. In all experiments data conformed satisfactorily to Michaelis-Menten kinetics, substituting serum concentrations for substrate concentrations (Fig. 2). The $V_{\max}$ values calculated for samples obtained on day 10 were about 30% higher than for those obtained on day 3 whereas apparent K_m values increased by about 50% between day 3 and day 10 (Table I). The apparent K_m values, i.e. the amount of serum necessary to provide half maximal reaction rate, were related to plasma concentrations of orosomucoid, apo-AI and HDL cholesterol.

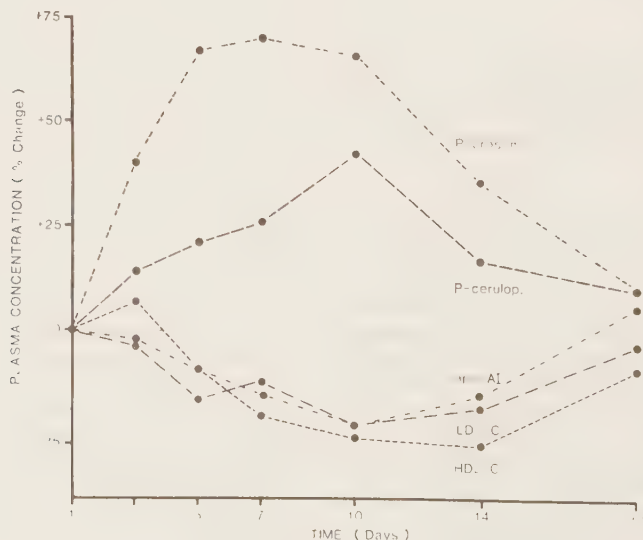


Fig. 1. Relative changes in the plasma concentrations of acute phase proteins, lipoproteins and apolipoprotein AI after myocardial infarction. Initial mean values $\pm$ SEM were plasma orosomucoid, 1.06 ± 0.07 g/l; ceruloplasmin 0.42 ± 0.02 g/l; apolipoprotein AI, $103 \pm 4.7\%$; HDL cholesterol, 0.99 ± 0.09 mmol/l; and LDL cholesterol, 4.28 ± 0.31 mmol/l.

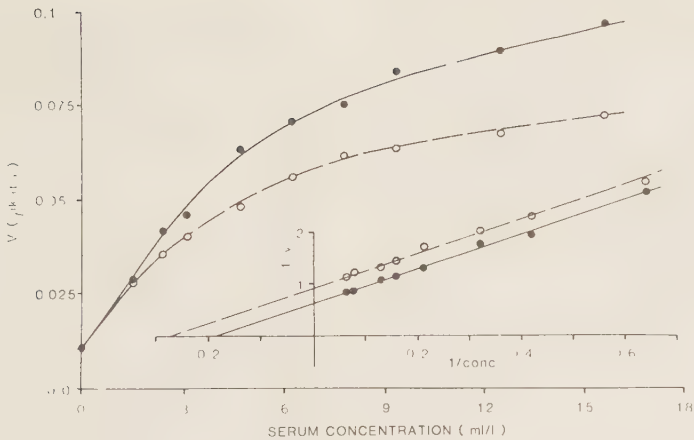


Fig. 2. Enzymatic activity of purified lipoprotein lipase in the presence of increasing concentrations of serum from a patient with myocardial infarction. Serum samples obtained on day 3 (○) and 10 (●) after onset are compared. Insert shows double reciprocal plot.

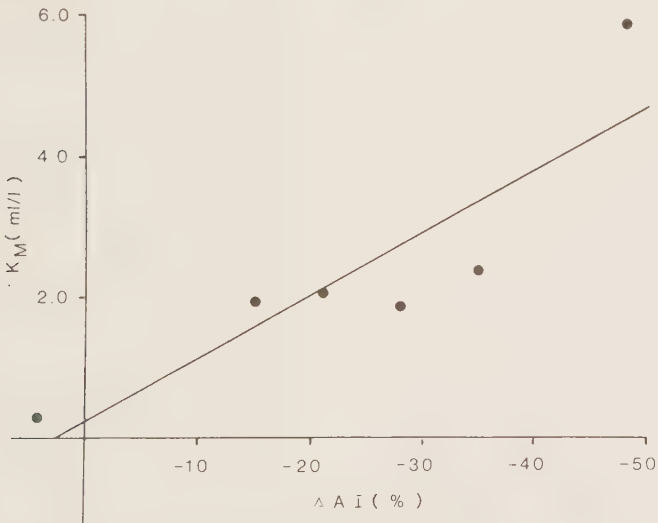


Fig. 3. Linear regression of changes in apparent K_M values (see Fig. 2) and changes in apo-AI levels in six patients after myocardial infarction. Regression equation, $y = 0.09x + 0.22$; correlation coefficient (r) = 0.88.

The correlation between apparent K_M and orosomucoid were highly significant ($r = -0.97$ for samples obtained on day 3 and $r = -0.82$ for samples drawn on day 10). There was no significant correlation between changes in P-orosomucoid levels and apparent K_M .

An even stronger relationship was found between apparent K_M and plasma HDL concentrations, especially when expressed as apo-AI levels. The correlation coefficients were -0.65 (day 3) and -0.84 (day 10). Furthermore, the changes in apparent K_M were significantly

TABLE 1. LPL activating ability of sera obtained 3 and 10 days after myocardial infarction*

	Day 3 (mean $\pm$ SD)	Day 10 (mean $\pm$ SD)	P
$V_{\max}^{\dagger}$	0.10 ± 0.03	0.14 ± 0.05	< 0.05
Apparent $K_m^{\ddagger}$	4.7 ± 1.6	7.1 ± 3.2	< 0.05

*Purified LPL was incubated with triacylglycerol substrate in the presence of increasing amounts of serum obtained from patients, 3 and 10 days after myocardial infarction. Data are given as mean $\pm$ SD

$\dagger \mu\text{kat/l}$.

$\ddagger$ Designates the concentration of serum (ml/l) necessary to provide half maximal reaction rate.

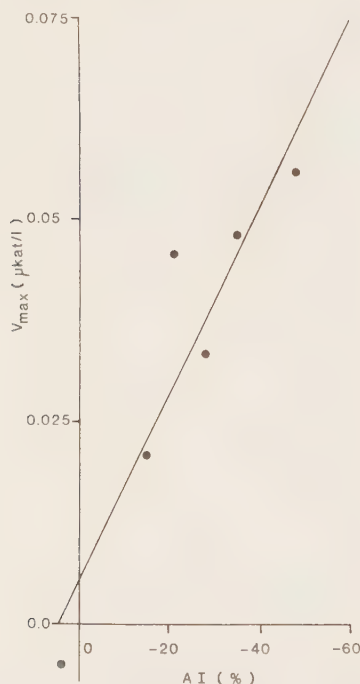


Fig. 4. Linear regression of changes in $V_{\max}$ values and changes in apo-AI levels in six patients after myocardial infarction. Regression equation, $y = 0.01x + 0.03$; correlation coefficient (r) = 0.93.

correlated to the changes in apo-AI (Fig. 3) and HDL cholesterol ($r=0.88$ and 0.79 , respectively). The concentrations of LDL cholesterol or P-triglyceride were not correlated to the apparent K_m values.

The absolute $V_{\max}$ values were not correlated to concentrations of plasma lipoproteins or acute phase proteins. However, the change in

$V_{\max}$ was significantly correlated to alterations in apo-AI (Fig. 4) and HDL cholesterol concentrations ($r=0.93$ and 0.94 , respectively). There were no relationships between $V_{\max}$ values and LDL cholesterol or plasma triglyceride concentrations.

DISCUSSION

The extent and time-course for the transient decrease in HDL concentrations, measured as HDL cholesterol or apo-AI, are consistent with earlier findings [8]. The present study demonstrates that LDL concentrations decrease transiently with a time-course essentially similar to that of HDL. VLDL concentrations, on the other hand, seem more constant after AMI; plasma triglyceride levels, under these conditions reflecting essentially VLDL, changed only insignificantly.

The mechanisms behind these transient effects on plasma lipoprotein levels have not been settled. Theoretically, the plasma concentration of any substance is determined by its rate of production and elimination; for the lipoproteins, another important determinant is the interconversion of lipoproteins in plasma [14]. Finally, plasma concentrations may be influenced by the distribution of lipoprotein particles between the intra- and extravascular space. An increased permeability of the capillary membranes during the acute inflammatory phase after AMI would conceivably lead to an extravasation of smaller plasma proteins; a similar mechanism is believed to account for the transient decrease in plasma albumin concentrations after surgical trauma [13].

HDL has molecular dimensions in the same order of magnitude as serum albumin (diameter 7–10 nm compared with 15×3.7 nm for albumin). The significant correlation found between the inflammatory response (i.e. increase in plasma orosomucoid) and the decrease in HDL concentrations is consistent with the view that the decrease in HDL may be caused by extravasation. In contrast, other mechanisms seem to be involved in the decrease in LDL concentrations seen after AMI, since there was no correlation between the decrease in HDL and LDL concentrations, and no relation between the inflammatory response and LDL concentrations. The reason for the transient decrease in LDL remains speculative.

In the present study we also tried to cast light upon the possibility that the altered plasma lipoprotein profile after AMI might in turn affect the degradation rate of VLDL to LDL. Since this process is associated with the formation of HDL [14], such a mechanism might further modify the HDL concentrations. The key enzyme responsible for the successive degradation of VLDL is LPL. This enzyme is dependent upon serum for optimal activity [14], and assays for LPL activity are generally performed in the presence of low concentrations of normal serum (10–20 ml/l) to provide maximal enzyme activity. *In vitro* experiments with purified apolipoproteins have demonstrated that the serum activation can be accounted for by the content of apo-CII. The exact mode of action of the activator is complex and still incompletely defined; effects on the enzyme, on the accessibility of the triglyceride substrate of the lipoprotein particle, on the affinity between enzyme and substrate and on product elimination from the reaction site have been suggested [14]. Apo-CI, apo-CIII and apo-E, on the other hand, can reverse the apo-CII-induced activation by unknown mechanisms [4, 7, 16]. HDL is regarded as a reservoir for these apolipoproteins *in vivo*; they can transfer to VLDL and chylomicrons to allow proper action of the LPL enzyme [14]. Therefore, changes in HDL concentrations, as occurring after AMI, may also affect LPL activity and the degradation rate of VLDL. To test this possibility, we have compared the activation characteristics of sera obtained initially during the study and at day 10, when the lowest HDL concentrations were recorded. Orosomucoid levels were also included in these analyses since it has been demonstrated to affect LPL activity and lipoprotein metabolism in the rat [19].

The activation data for all serum samples conformed satisfactorily to Michaelis–Menten kinetics and allowed the calculation of maximal reaction rate and apparent K_m , substituting serum concentrations for substrate concentrations. The transient decrease in lipoprotein concentrations was associated with marked changes in the activation pattern: sera drawn from the patients on day 10 after AMI could, when added in saturating amounts, enhance LPL activity better than sera obtained on day 3; on the other hand sera from day 3 were more

efficient in activating LPL at suboptimal concentrations, and significantly larger amounts of the sera from day 10 were needed to provide half-maximal reaction rates. Our limited knowledge of the mechanisms for the interaction of LPL, triglyceride substrate, activating apo-CII, inhibitory apolipoproteins and possibly other effectors [14] makes it difficult to speculate on the physiological implications of these data. However, the strong relationships between alterations in HDL concentrations and LPL activation indicate that the concentrations and proportions of various apolipoproteins may be of importance for the rate of the LPL reaction under physiological or pathophysiological conditions. In contrast, earlier *in vitro* studies, using the perfused rat heart, have suggested that the concentrations of apo-CI, -CII and -CIII are normally not rate-limiting or regulatory in VLDL degradation [5, 12]. Also, the striking correlations between the concentrations of orosomucoid on one hand and V_{max} and K_m values on the other, indicates that this plasma protein may have important modulating effects on the LPL reaction under physiological conditions [19].

It is interesting to note certain congruities between the present data and earlier *in vitro* studies on the individual apolipoproteins and LPL. For example, V_{max} increased (i.e. the LPL reaction was enhanced) when HDL concentrations decreased on day 10; V_{max} , therefore, seems to reflect mainly the influence of LPL-inhibiting apolipoproteins. This interpretation is consistent with the fact that when tested *in vivo* [4], the effect of these inhibitory apolipoproteins is most prominent at relatively high concentrations and in systems where apo-CII is present at saturating concentrations. In contrast, the apparent K_m values increased (i.e. the affinity between substrate and enzyme seemed to decrease) when HDL levels decreased. This variable thus appears to reflect mainly the influence of the LPL activating apo-CII. Again, this interpretation is consistent with *in vitro* studies [4] showing that only apo-CII has effects on the LPL reaction when added at low concentrations.

Thus, the changes in plasma lipoprotein concentrations after AMI seem to result from a complex interplay between several pathogenetic mechanisms. We suggest that extravasation may be one important factor at least

for the smaller HDL lipoproteins, whereas the contribution of other mechanisms such as altered patterns of intravascular metabolism (as suggested by the present data) or altered rates of elimination of different lipoprotein particles remains to be further clarified.

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II.

IN-HOSPITAL EXERCISE THERAPY IN PATIENTS WITH SEVERE ANGINA PECTORIS

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ABSTRACT

We have evaluated physiological and metabolic effects of a nine weeks' in-hospital training program in fourteen males with severe, disabling angina pectoris. The exercise program consisted of two 30 min sessions daily of intensive interval training on an ergometer bicycle. The physical performance increased by about 40% ($p < 0.001$). Plasma insulin levels were reduced and glucose tolerance improved significantly. There was a decrease in plasma triglyceride and LDL cholesterol levels, but no changes occurred in HDL cholesterol, apolipoprotein AI and B concentrations. Plasma triglyceride ($p < 0.05$) and LDL cholesterol ($p < 0.05$) levels remained low 3 weeks after completion of the training period and the physical performance remained improved ($p < 0.01$) even 6 months post-training. Four of the patients, who had been disabled for at least 5 months, were able to return to work.

We suggest that comparatively short and intensive in-hospital rehabilitation of patients with coronary heart disease may be an attractive alternative to prolonged training on an out-patient basis, especially in patients with severe angina pectoris.

INTRODUCTION

Physical training programs improve exercise tolerance and elevate the pain threshold in patients with angina pectoris (2, 4, 14, 23). Such training has also positive metabolic effects, especially on glucose tolerance and insulin sensitivity (1, 25).

Recently, considerable interest has focused on the effects of physical exercise on plasma lipoproteins. Low density lipoprotein (LDL) concentrations generally decrease during training (16, 22) and several studies indicate that high density lipoprotein (HDL) levels may increase after physical fitness programs (10, 15, 28). Such information is of special interest in relation to conditioning of patients with cardiovascular disease, since HDL and LDL levels seem to have a direct influence on the atherogenetic process (21). Patients with coronary heart disease often have moderate changes in their lipoprotein profile, with comparatively low HDL and slightly elevated LDL levels (5, 6).

Earlier studies in this area have generally dealt with the effects of long-term physical rehabilitation in out-patients with mild to moderate angina pectoris, especially after acute myocardial infarction (1, 3, 8, 10). In the present investigation we have followed the physiological, psychological and metabolic effects of an intensive exercise program of comparatively short duration in a group of in-patients with severe, stable angina pectoris.

TABLE I. Clinical data

Patient No	Age (years)	pre-training	Body weight (kg) post-training	after 6 months
1	57	98.5	94.5	95.0
2	53	74.5	73.0	76.0
3	55	60.5	60.0	63.0
4	44	73.5	73.5	74.0
5	57	76.0	77.0	-
6	52	87.0	84.5	93.0
7	49	95.5	86.2	87.0
8	46	85.0	81.0	80.0
9	53	77.0	77.0	76.0
10	47	77.0	77.0	79.0
11	58	82.0	81.0	84.0
12	46	82.0	79.5	82.0
13	57	92.0	87.5	90.0
14	56	79.0	79.0	79.0
mean $\pm$ S.D.		81.4 $\pm$ 9.9 ¹⁾	79.3 $\pm$ 8.0	81.4 $\pm$ 8.6

1) 81.8 $\pm$ 10.2 when patient no. 5 is excluded.

METHOD

Patients

Fourteen men, mean age 52 years, with stable angina pectoris participated in the study (Table I, II). They were all severely disabled and were offered physical training as a complement to maximal pharmacological therapy and in some instances as an alternative for coronary surgery. The diagnosis of angina pectoris was based on marked ST segment changes in the resting ECG (Table I, II) or on typical chest pain and characteristic ECG changes (at least 2 mm horizontal ST segment depression) during exercise testing. All but two patients had suffered previous myocardial infarction (Table I, II). None of the patients had diabetes or laboratory evidence of liver disease.

Medications with digitalis, beta-blockers and Ca-antagonists (Table I, II) were kept unchanged throughout the study. Nitroglycerin consumption and the frequency of angina pectoris attacks were continuously registered.

All the participants had been heavy smokers but five stopped at the time of their first myocardial infarction. At the beginning of the study seven were still smoking, on the average six cigarettes per day (Table I, II). Smoking habits remained unchanged.

Three patients were slightly and two markedly overweight (Broca's index^{x)} 1,28 and 1,36).

$$x) \text{ Broca's index: } \frac{\text{body weight (kg)}}{\text{height (cm)} - 100}$$

(reference interval 0.8 - 1.2)

TABLE II. Clinical data

Patient No.	Time to latest myocard. infarction (months)	Cigarettes/day (n)	Medication	Comments
1	-	0	digoxin, verapamil	
2	84	0	verapamil	
3	7	0	metoprolol	
4	6	0	verapamil	
5	4	0	digoxin, metoprolol	1)
6	11	20	verapamil	1), wine regul. before
7	7	3	digoxin, metoprolol	1), 2)
8	5	1	digoxin, metoprolol	3)
9	12	5	atenolol	
10	-	0	atenolol	
11	11	0	verapamil	
12	48	1	metoprolol, verapamil	
13	10	0	digoxin, verapamil	
14	7	3	metoprolol	2), low sat. fat diet before

1) Marked ST changes on resting ECG

2) Moderately increased heart volume on chest X-ray

3) Prednisolone 10 mg/day due to post myocardial infarction syndrome

From the beginning of the rehabilitation program all patients were carefully informed about their disease in order to reduce fear and tension. The same information was also given to their families. Furthermore, social workers started immediately to make efforts to help the patients to return to their jobs.

One patient had been on a low saturated fat diet, the others ate an ordinary Swedish diet. None had regular drinking habits, except for one patient who used to consume about 750 ml wine daily. During the training program they all had an ordinary Swedish diet, and no alcohol was allowed.

No attempt was made to influence the patients' diet until the last week in hospital and after discharge from hospital. Five overweight patients were put on caloric reduction (1000 Kcal daily) and six patients with plasma cholesterol levels over 8.0 mmol/l were recommended a diet with a high proportion of polyunsaturated fat from the last week of the training program.

Training program

Physical working capacity was assessed by an exercise test carried out about 2 - 4 months before and 6 months after the end of the training program. The patients were tested on an electrically braked bicycle ergometer with step-wise increasing work loads, 6 minutes at each step, up to exhaustion, and the maximal pulse-rate was recorded. In all patients, especially before the training, angina pectoris was the limiting factor.

The training period comprised 9 weeks which were spent in a local Rehabilitation Center. The physical exercise consisted of a supervised interval program of cycling 30 minutes twice a day. One of the two bicycle sessions was supervised by a physiotherapist. The other session was organized by an occupational therapist using an "Acron bicycle saw". The bicycle runs a sawblade, with which the patient can cut out a jig-saw puzzle on a table in front of him. The patients also participated in half an hour's session of calisthenics, jogging and walking, the intensity of which was individually adapted.

The intensity of the cycling program was individually determined according to the maximal pulse-rate, as assessed in the previous exercise test. The work load was adjusted to give a training pulse-rate just below the maximal pulse-rate ("tolerated work load"). Each training session consisted of 3 minute periods of cycling at the tolerated work load alternating with 3 minute periods at half maximal work load for a total of 30 minutes.

In general, the tolerated work load increased continuously during the nine weeks training program, and the intensity of the training could be step-wise increased during the study. When a patient failed to improve his working capacity from one week to another, on a defined training pulse-rate, a new exercise test was carried out as described above. The training pulse-rate was then increased according to the new maximal pulse-rate.

No complications occurred during the training program. When leaving the study, the patients were encouraged to maintain regular physical activities, but no specific

home programs were prescribed.

Analyses

Blood samples were drawn after an overnight fast four weeks before, at admission, after three, seven and nine weeks of training, and again three weeks after return to home.

The concentrations of plasma orosomucoid, apolipoprotein AI and B were measured by electroimmunoassay (18). Data for the apolipoproteins are expressed as percent of the value of a plasma pool obtained from 40 healthy males. The oral glucose tolerance test, carried out during the first and seventh week of training, was performed with 30 g glucose per m^2 body surface area given as an aqueous solution (10 g/100 ml) and ingested within 5 minutes. Venous blood samples of blood glucose (20) and plasma insulin (12) concentrations were followed during 2.5 hours after glucose intake.

Plasma cholesterol (24) was measured enzymatically with reagents from Boehringer Mannheim, West Germany. The fraction of plasma cholesterol associated with the high density lipoproteins (HDL cholesterol) was quantitated by precipitation of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) by $Mg\ Cl_2$ and dextrane sulfate (7) with subsequent determination of cholesterol in the clear supernatant. LDL cholesterol was estimated from the equation: $LDL\ cholesterol = plasma\ total\ cholesterol - [(plasma\ triglycerides \times 0.45) + HDL\ cholesterol]$; all values in mmol/l (11). Plasma triglycerides were determined enzymatically (26).

The significance of changes in different variables was evaluated using Student's t-test. Correlations between different variables were analyzed by linear regression analyses.

TABLE III. Physical working capacity before and six months after training

Patient No.	Pre-training (W)	Post-training (W)
1	115	115
2	67	117
3	70	97
4	58	120
5	60 ¹⁾	-
6	100	120
7	60	85
8	80	93
9	54	72
10	108	133
11	110	112
12	n.d. ²⁾	n.d.
13	90	110
14	70	80
mean $\pm$ S.D.	81.8 $\pm$ 21.9	104.5 $\pm$ 18.8 ^{xx}
n	12	12

1)
Not included in calculation of mean

2)
n.d., not determined

xx = $p < 0.01$

RESULTS

The mean body weight decreased on the average by 2 % during the training program ($p < 0.01$). Six months after leaving the study the body weight had again returned to initial values (Table I, II). The weight reduction was most pronounced in the overweight patients.

Before training the patients had a low physical working capacity (Table III). In all cases the exercise test was interrupted due to angina pectoris.

"Tolerated work load", as defined above, was low during the initial part of the study. It increased in all subjects, except for no. 5, on the average by 41% ($p < 0.001$), during the training program (Table IV).

The fact that the patients did not report any change in the frequency of angina pectoris attacks or nitroglycerin consumption can be explained by the continuous increase of the work load during the training program.

The improved physical performance was essentially maintained six months after the termination of the rehabilitation program (Table III). Mean physical working capacity was then still 28% above pre-training levels ($p < 0.01$), and although most of the patients still interrupted the exercise test due to chest pain the pain threshold was much higher than before training.

The two methods to evaluate physical performance (i.e. physical working capacity and "tolerated work load"), were highly correlated (correlation coefficient, 0.89; $p < 0.001$).

TABLE IV. Intensity of the training program.

Patient No.	"Tolerated work load" (W)	
	week 1 - 2	week 8 - 9
1	100 ¹⁾	120
2	75	104
3	60	95
4	50	75
5	50	50
6	70	85
7	50	75
8	70	105
9	55	70
10	90	155
11	90	95
12	75	100
13	70	120
14	45	90
mean $\pm$ S.D.	68 $\pm$ 17.2	96 $\pm$ 25.8 ^{xxx}
n	14	14

1)

Mean of the tolerated work load during the first two weeks of training

xxx = $p < 0.001$

Three weeks after completion of the supervised training the patients were interviewed about the impact of the program on their experience of work tolerance, angina pectoris, fear and tension and cardiac handicap. All patients reported positive effects of the program, and in one patient the feeling of a cardiac handicap had totally disappeared. Thirteen patients reported improved physical performance, 8 experienced less fear and tension, and 3 subjects had noticed markedly reduced angina pectoris.

Three months after the end of the study four patients, who had not been able to work for at least five months prior to training could take up suitable jobs. Two went back to their earlier work. Five patients already had or had recently been recommended some kind of pension. Two were still disabled at the end of the study but hoped to be able to work in the future. One patient (No. 5) died of myocardial infarction 4 weeks after the discharge from the Rehabilitation Center.

The glucose tolerance improved in all patients except subject no. 6, who had previously been a regular wine consumer (Fig. 1).

There was no consistent relation between the improved glucose tolerance and the increase in "tolerated work load". However, a tendency toward significant correlation was found between the increase in "tolerated work load" and the change in glucose values during the initial part of glucose tolerance test (correlation coefficient, -0.51 for the glucose values at 30 minutes).

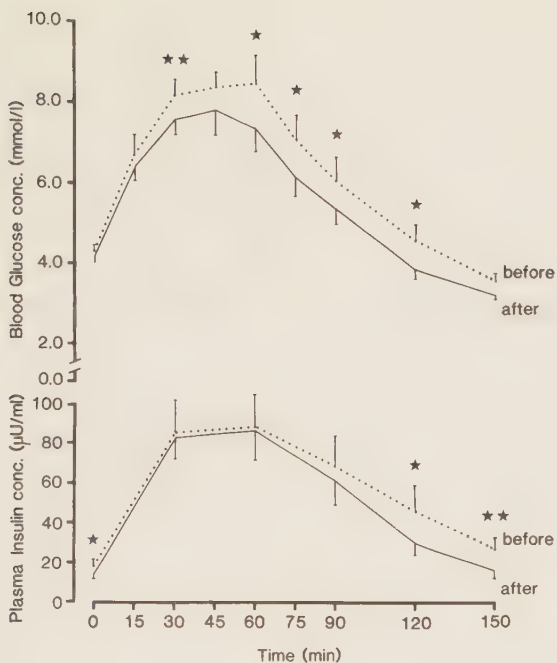


Fig 1. Mean plasma insulin and glucose values during oral glucose tolerance test in 14 patients with severe angina pectoris before and after seven weeks of physical training. Vertical bars denote standard error of the mean (S.E.M.)

* = $p < 0.05$, ** = $p < 0.01$

The fasting plasma insulin concentrations were also significantly reduced (Fig. 1). Insulin levels were also lower after glucose ingestion, especially during the later phase of the glucose tolerance test (Fig. 1).

Before training mean plasma cholesterol and mean plasma triglyceride levels were both around the upper reference limit, 7.1 ± 1.4 mmol/l and 2.3 ± 1.4 mmol/l, respec-

tively.

The initial mean HDL cholesterol concentration was comparatively low, (0.86 ± 0.20 mmol/l; lower reference limit in our laboratory is 0.80 mmol/l) and LDL cholesterol was high (5.4 ± 1.5 mmol/l; upper reference limit 4.5 mmol/l) giving an elevated LDL/HDL ratio of 6.5. The mean apolipoprotein AI value was $85 \pm 13\%$ before training and that of apolipoprotein B $118 \pm 18\%$.

Mean LDL cholesterol concentrations (Fig. 2) decreased after 3 weeks exercise with 17% ($p < 0.01$) and remained 10 - 15% below initial values during the rest of the study. The plasma concentrations of HDL cholesterol,

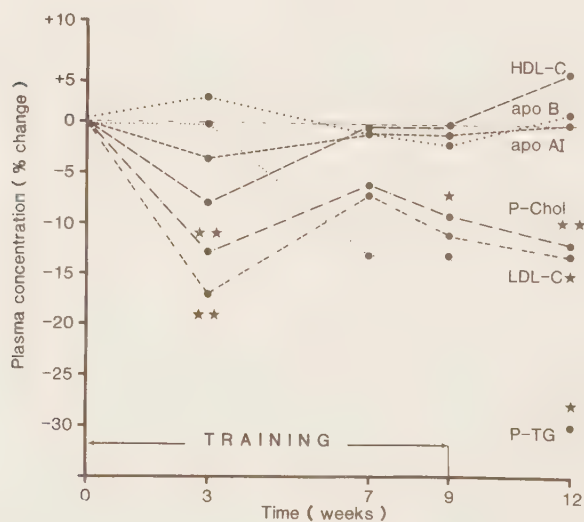


Fig 2. Relative changes of mean plasma concentrations of plasma cholesterol, tri-glycerides, HDL cholesterol, LDL cholesterol, Apo AI and Apo B during and 3 weeks after training.

apolipoprotein AI and B did not change during training (Fig. 2), but the LDL/HDL ratio was reduced by 12% (n.s.). The improvement of the lipoprotein profile was maintained at the visit to the out-patient clinic three weeks after the end of the training period (Fig. 2).

The decrease in LDL cholesterol concentrations, from the first to the seventh week, was significantly correlated to the improvement in plasma insulin levels both at the 60, 90 and 120 minutes measurements of the glucose tolerance test. The best correlation was found at 90 minutes correlation coefficient, 0.82 ($p < 0.01$) (Fig. 3).

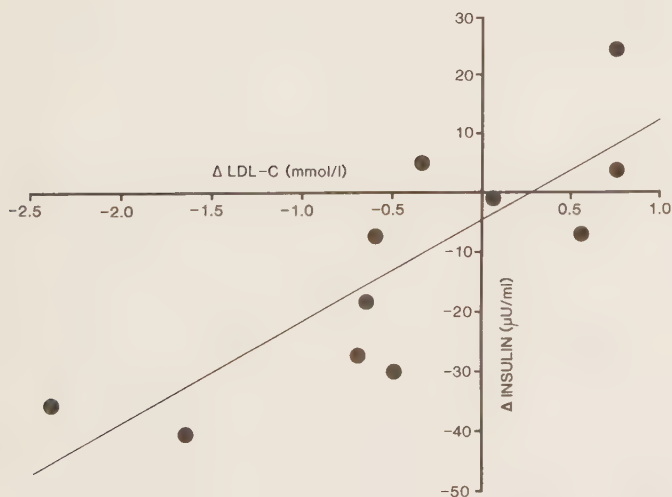


Fig 3. Linear regression of changes after 7 weeks of training in LDL cholesterol and plasma insulin concentrations at 90 minutes during oral glucose tolerance test. Regression equation, $y = 16.66 X - 4.76$; correlation coefficient, 0.82 ($p < 0.01$)

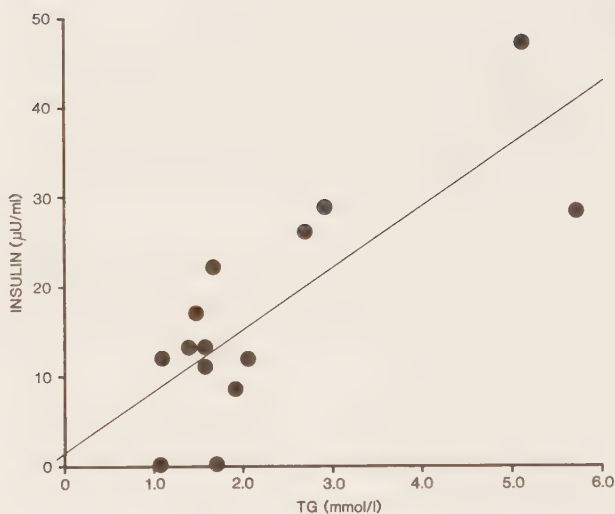


Fig 4. Linear regression of pre-training values in plasma triglyceride and insulin concentrations. Regression equation, $y = 6.89 X + 1.34$; correlation coefficient, 0.78 ($p < 0.001$)

Also plasma triglyceride levels were significantly correlated to plasma insulin levels, correlation coefficients 0.78 ($p < 0.001$) before training (Fig. 4), and 0.53 ($p < 0.05$) after 7 weeks exercise. The decrease in plasma triglyceride concentration from the first to the seventh week was significantly correlated to the decrease in plasma insulin levels measured before (Fig. 5) and 120 minutes after glucose ingestion (correlation coefficients, 0.70 and 0.65, respectively; $p < 0.05$).

Variables reflecting physical performance, i.e. physical working capacity and "tolerated work load" were not correlated to the concentrations of plasma lipoproteins and apolipoproteins.

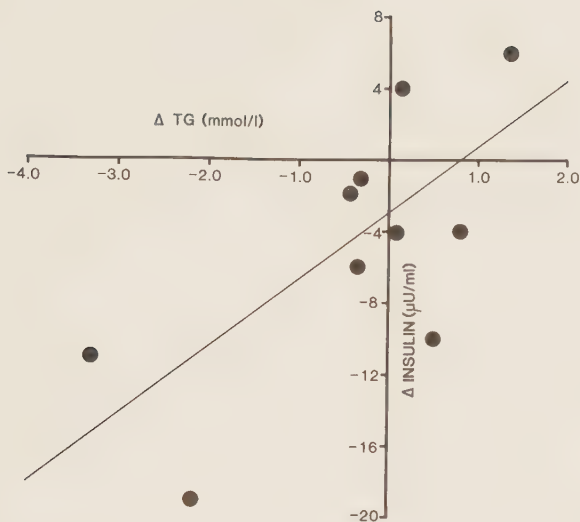


Fig 5. Linear regression of changes after 7 weeks of training in plasma triglyceride and insulin levels. Regression equation, $y = 3.74 X - 3.03$; correlation coefficient, 0.70 ($p < 0.05$).

DISCUSSION

The positive effects of physical conditioning in patients with CHD are well established (2, 14). Exercise therapy of patients with stable angina pectoris has clearly shown that the threshold for precipitation of chest pain can be elevated by training (4, 23). Long-term rehabilitation programs, often of mild to moderate intensity, have become an accepted form of therapy, especially after myocardial infarction. The aim of such a program is generally to enhance the patients physical performance as well as to improve his emotional state and facilitate his return to work. For economic reasons out-patient training once to three times a week is, generally, recommended in the first place. However, the drop-out rate, especially of severely disabled patients is often high in such programs (3, 27).

In the present study we have tried to elucidate if an intensive, comparatively short, in-hospital exercise program in patients with severe, stable angina pectoris has similar beneficial effects on physiological, psychological and metabolic variables as reported in long-term, out-patient training.

Our results regarding psychosocial factors and return to work are similar to those reported in earlier studies (13, 17). Also, it is evident that the physical performance of our subjects improved to about the same extent as reported after long-term programs. The mean physical working capacity recorded six months after completion of the present rehabilitation program, 105 W, is about 70% of that in healthy subjects of the same age. These

results compare favorably with those obtained by other training programs in patients with severe angina pectoris (9). The reason why patient no. 5 did not improve his physical performance is explained by the fact that he had had three large myocardial infarctions before he was considered a candidate for the present program. He also was the most disabled of the patients and had very frequent anginal attacks. His death occurred 4 weeks after the completion of the training and was not due to any complication of the training program as such.

The decrease in plasma insulin levels in combination with an improved glucose tolerance after training (c.f. 19) suggests an increased peripheral insulin sensitivity (1) possibly mediated by an increase in the number of insulin receptors (25).

In our series, like in others (5, 6), an overrepresentation of deranged plasma lipoprotein patterns were found before training. Most frequently, HDL levels tended to be low and LDL levels to be elevated. The most prominent alteration, during the physical exercise program, was a significant decrease of plasma LDL which still persisted three weeks after the training (16, 22). With the specific design of the study as to intensity, frequency and duration of the training period, no changes in HDL cholesterol were registered (22). However, the LDL/HDL ratio was considerably improved after the training program.

In summary, this study shows that a relatively brief training program of high frequency and intensity gives results comparable to those reported in traditional out-patient training programs with regard to physiolog-

ical, psychological and metabolic variables. Particularly in patients with severe angina pectoris, who usually have a high drop-out rate from exercise programs (3, 27) an in-hospital training regime of this kind seems to be an important complement to pharmacological therapy. It can also be recommended as an alternative to coronary by-pass surgery in specially selected patients.

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III.

PLASMA LIPOPROTEINS AND LIPOLYTIC ENZYME ACTIVITIES DURING ENDURANCE TRAINING IN SEDENTARY MEN: CHANGES IN HDL SUBFRACTIONS AND COMPOSITION

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ABSTRACT

Eighteen healthy, sedentary males took part in supervised bicycle training 50 min three to five times a week. Twelve subjects (group A) trained for 6 weeks at heavy and 6 subjects (group B) for 12 weeks at moderate intensity. Maximal oxygen uptake increased by about 20% ($p < 0.01$). Body weight and composition as well as diet remained unchanged.

After six weeks plasma high density lipoprotein (HDL) cholesterol concentrations had increased by 7% ($p < 0.05$) in all subjects together. The increase was most marked in group B, 14% ($p < 0.05$) compared to 3% in group A (ns). Apolipoprotein AI increased by about 7% in both groups ($p < 0.01$). After 12 weeks HDL cholesterol and apo AI levels had almost returned to initial values.

Measurements of HDL components showed increases of 6-12% in free cholesterol, cholesteryl ester ($p < 0.05$) and phospholipid ($p < 0.01$) whereas the minor triglyceride fraction decreased by 20% ($p < 0.01$). Zonal ultracentrifugation, in 4 subjects, revealed a preferential rise of about 35% in the HDL₂ subfraction, increasing the HDL₂/HDL₃ ratio by about 20%. In parallel the composition of the lipoprotein classes changed: the protein moiety of all classes, except LDL, expanded at the expense of the core components, cholesteryl ester and triglyceride.

Hepatic lipase activity decreased by 6% ($p < 0.05$) and lipoprotein lipase (LPL) activity in adipose tissue increased by about 50% ($p < 0.05$) while LPL activity in postheparin plasma and skeletal muscle did not change.

The alterations in HDL concentration were related to changes in body composition and diet, especially fat ingestion. Correlations between HDL levels, LPL and HL activities indicate that the effect of training on HDL is partly mediated via these enzymes.

INTRODUCTION

Several studies have shown an inverse relationship between high density lipoproteins (HDL) and coronary heart disease (CHD) (1, 2, 3). HDL levels are higher in physically active than in sedentary men (4, 5), but in longitudinal studies an effect of physical training on HDL levels has been more difficult to establish. While some authors have reported significant increases in HDL concentrations during physical conditioning (6, 7, 8, 9, 10) others have not been able to do so (11, 12, 13).

These contradictory results might be explained by differences in training intensity and duration. Also, variable effects of the training programs on caloric intake and diet composition and on body weight might contribute to the variability in the metabolic effects of training.

The aim of the present study was therefore to investigate the effects of training of varied intensity and duration on plasma lipoproteins and to relate these effects to changes in diet and body weight and composition. To study possible mechanisms behind the changes in lipoproteins, we have also measured the activities of two key enzymes in lipoprotein metabolism, i.e. lipoprotein lipase (LPL) and hepatic lipase (HL).

MATERIALS AND METHODS

Subjects

Eighteen healthy males, mean age 41 (range 32 - 53) years, were recruited by advertising in the local hospital bulletin. The protocol, approved by the local ethics committee, was explained in detail to the subjects who gave their formal consent. All were hospital employees with sedentary jobs. They had normal blood pressure, normal resting ECG and normal ECG reaction during a maximal exercise test. All subjects had plasma cholesterol and triglyceride levels within the reference range and none took any medication. All participants had ordinary Swedish diet habits and none had a history of excess alcohol consumption. They were encouraged to keep diet, drinking and smoking as constant as possible throughout the study.

The volunteers were allocated to two groups. Group 'A' included twelve and group 'B' six subjects. The groups did not differ in respect to age, physical fitness, alcohol intake, diet and body weight. Two subjects in group 'A' and one in group 'B' were overweight (Broca's index ^{x)} 1.35, 1.28 and 1.28). Four subjects in group 'A' and one in group 'B' were smokers, smoking on the average 14 cigarettes per day. Plasma cholesterol, low density lipoprotein (LDL) cholesterol and triglyce-

$$x) \quad \text{Broca's index: } \frac{\text{body weight (kg)}}{\text{height (cm)} - 100}$$

(reference interval 0.8 - 1.2)

ride levels were essentially identical in both groups. The average initial high density lipoprotein concentrations were higher in group 'A' (HDL cholesterol 1.26 mmol/l vs. 1.08 mmol/l, apolipoprotein AI 103% vs. 98%), but these differences did not reach statistical significance.

Body composition

In group 'A' total body potassium was estimated before and after six weeks of training by measurement of ^{40}K in a whole body scintillation counter calibrated with a phantom. Lean body mass (LBM) was determined according to Forbes (14). Body fat weight was calculated by subtracting lean body mass from the body weight.

Diet

Dietary habits were registered by a clinical nutritionist, who obtained a careful 'diet history' according to Burke (15, 16) before and after six weeks in all subjects and in group 'B' also after twelve weeks. The Food Composition Tables edited by the Swedish National Food Administration were used to calculate the dietary content of fat, protein and carbohydrates.

Training

Group 'A' took part in six weeks of training three times a week. Group 'B', including six men, was formed later on to polarize the material by decreasing the training intensity, increasing the length of the training period to twelve weeks and, in three subjects, increasing the number of training sessions from three to five per week.

Each training session, identical in both groups, consisted of 50 min supervised cycling on a bicycle ergometer. Five min of warming up was followed by 40 min of cycling during which the work-load was adjusted to give heart-rates of at least 85% (group A) and 70% (group B) of the maximal heart-rates. The session was ended by five min of slowing down.

Mean attendance was 96% and there were no drop-outs during the training period.

Individual training pulses were continuously registered and used to estimate the intensity of exertion (17) and energy expenditure (18) during the program. Group mean values of these variables are shown in Table 1. The training intensity differed significantly between the groups.

Exercise test

Exercise tests were performed before and at three weeks intervals during the training period. The tests were

performed on electrically braked bicycle ergometers. Starting at 50 W the work-loads were stepwise increased by 50 W every 6 min up to exhaustion.

Maximal oxygen uptake (VO_2 max) was estimated from work-load at heart rate 170/min (or below) using the Åstrand nomogram (19).

Blood sampling

Blood samples were drawn between 7 and 8 a.m. 2-3 days after the latest training session and after the subjects had been fasting for at least 10 h. Initial values represent the mean of two samples drawn within a week before the start of the study.

During training blood samples were drawn at two weeks intervals. In nine subjects blood samples were also taken six weeks after cessation of the training program.

Biopsies

Tissue biopsies were performed in the subjects in group A between 7 and 8 a.m. twice within one week before and once after the training period, 48 - 72 h after the last training session. Initial values represent the mean of the two pre-training measurements. Subcutaneous adipose tissue was taken from the gluteal region by needle aspiration (20). Skeletal muscle was obtained from vastus lateralis muscle at the lateral mid point

of the thigh with a Bergström needle (21). The biopsy specimens were rinsed in physiological saline, weighed and assayed immediately.

Laboratory analyses

Plasma cholesterol and triglycerides were determined enzymatically with reagents from Boehringer Mannheim, West Germany (22, 23). High density lipoprotein (HDL) was quantitated and characterized in the supernatant obtained after precipitation of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) by $MgCl_2$ and dextrane sulphate (24). The free and esterified fractions of HDL cholesterol were differentiated by performing the measurement with and without the cholesterol esterase reagent. HDL phospholipids were determined as lipid phosphorus. LDL cholesterol was calculated according to the formula of Friedewald (25).

The concentrations of apolipoprotein AI were measured by electroimmunoassay (26). Data for the apolipoproteins are expressed as per cent of the value of a plasma pool from 40 healthy males.

Separation of plasma lipoproteins into lipoprotein classes and HDL subfractions by zonal ultracentrifugation was performed in blood samples from four subjects in group A before and after six weeks of training essentially as described earlier (24). The absorbance at 280 nm of the eluent from the rotor was monitored, and the areas of the peaks of the lipoprotein classes measured by planimetry. The composition of the lipoprotein clas-

ses was determined in representative pools from each lipoprotein fraction by the methods described above; protein was measured by a scaled-down version of the method described by Lowry et al (27).

Lipoprotein lipase (LPL) and hepatic lipase (HL) activities were determined in postheparin plasma drawn 10 min after the injection of 100 U heparin/kg body weight with methods described earlier (28). LPL activity in adipose tissue and skeletal muscle specimen was determined after elution with heparin (28, 29).

Statistical analyses

The significance of changes in different variables was evaluated using Wilcoxon's rank order test for paired observations. Correlations between different variables were analyzed by linear regression analysis.

TABLE 1

Training intensity and change in maximal oxygen uptake (VO_2) max before and after six weeks (mean $\pm$ SD).

Group	Training pulse (beats/min)	Training pulse in % of max pulse	% VO_2 max a)	Energy expenditure b) (KJ/session)	Total energy expenditure during six weeks (MJ)	VO_2 max before (ml/kg/min)	VO_2 max after (ml/kg/min)
A	155 $\pm$ 8	89 $\pm$ 3	85 $\pm$ 4	2418 $\pm$ 442	36.6 $\pm$ 6.5	33 $\pm$ 7	41 $\pm$ 7 f)
B	140 $\pm$ 8 e)	77 $\pm$ 2 e)	68 $\pm$ 4 e)	1862 $\pm$ 325 e)	30.8 $\pm$ 2.1 c) 57.2 $\pm$ 17.6 d)	36 $\pm$ 10	39 $\pm$ 7 g)
A+B	150 $\pm$ 11	85 $\pm$ 6	79 $\pm$ 9	2233 $\pm$ 482	39.1 $\pm$ 11.8	34 $\pm$ 8	40 $\pm$ 8 f)

a) Calculated according to Saltin (17)

b) Calculated according to Shepard (18)

c) Three times a week

d) Five times a week

e) Significantly different from group A ($p < 0.01$)

f) Significantly different from VO_2 max before ($p < 0.01$)

g) Significantly different from VO_2 max before ($p < 0.05$)

RESULTS

Physical performance

Group mean values of estimated VO_2 max before and after six weeks of training are given in table 1. The difference in initial VO_2 max values between the groups did not reach statistical significance.

VO_2 max increased on the average by 19% ($p < 0.01$) after six weeks of training. It increased more rapidly in members of group A, who trained at a higher intensity and who had slightly lower initial levels. At three weeks it was 16% ($p < 0.01$) and at six weeks 23% ($p < 0.01$) above pre-training levels compared to 5% and 10%, respectively, in group B. In group B VO_2 max continued to increase with continuing training and after twelve weeks it was 18% above initial values ($p < 0.05$). Six weeks after completion of the training VO_2 max levels had dropped to levels 9% above the initial.

Diet and smoking

The initial average energy intake was $10\ 902 \pm 2\ 744$ KJ (mean $\pm$ S.D.) per day and consisted of $16 \pm 3\%$ protein, $35 \pm 6\%$ fat, $47 \pm 8\%$ carbohydrates and $3 \pm 4\%$ alcohol. The average change in mean energy intake and food composition, during the first six weeks of training was less than 1% but there was a great individual variation (from $- 2\ 217$ to $+ 2\ 288$ KJ). Also, the relative intake of fat

varied considerably (from - 9 to + 8%). The mean alcohol intake decreased by 3% (n.s.). There was no significant change in smoking habits.

Body weight and body composition

Mean body weight before training was 81.2 ± 10.9 kg and had decreased by 0.5% (n.s.) after six weeks. In group B it was 0.9% (n.s.) below initial values after twelve weeks of training.

LBM was 57.5 ± 5.0 kg before training and 58.3 ± 4.8 kg after six weeks. Per cent body fat decreased from 28.3 ± 8.2 to $27.4 \pm 7.8\%$. Neither of these changes reached statistical significance.

Plasma lipoproteins and apolipoprotein AI

Plasma triglyceride concentrations showed a transient increase in both groups. In group A it was 0.30 mmol/l (25%) ($p < 0.05$) higher after two weeks than before training (Fig 1), and in group B it was 0.13 mmol/l (11%) (n.s.) above pre-training levels after four weeks (Fig 2). The triglyceride levels then dropped again to baseline values after 6 weeks. In group B it was 0.15 mmol/l (12%) (n.s.) below initial levels after twelve weeks (Fig 2).

Plasma LDL cholesterol concentrations decreased during the exercise program. After six weeks it was 0.23 mmol/l

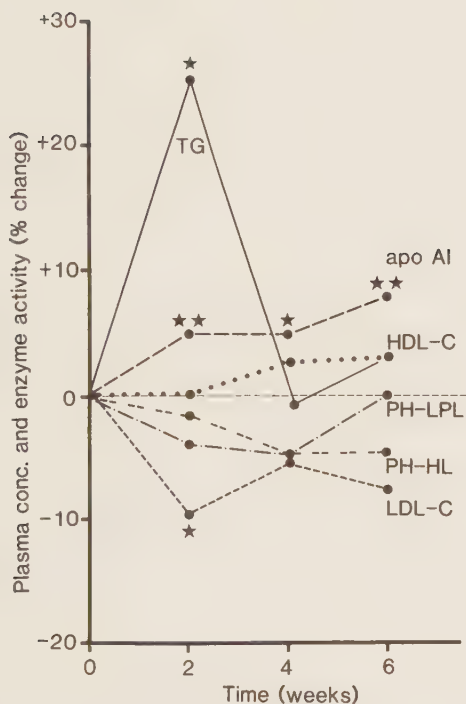


Fig 1. Relative changes in plasma lipoprotein and apo AI concentrations and heparin releasable lipolytic enzyme activities during 6 weeks of training in 12 subjects (group A). Initial values were (mean $\pm$ S.D.): Triglycerides (TG) 1.2 ± 0.5 mmol/l, LDL cholesterol (LDL-C) 3.8 ± 0.9 mmol/l, HDL cholesterol (HDL-C) 1.26 ± 0.29 mmol/l, apo-lipoprotein AI (apo AI) $103 \pm 13\%$, lipoprotein lipase activity (LPL) 1.26 ± 0.65 μ kat/l and hepatic lipase activity (HL) 5.24 ± 2.96 μ kat/l. * = $p < 0.05$, ** = $p < 0.01$.

(6%) lower than at the beginning. In group B, who trained for twelve weeks, the decrease in LDL cholesterol reached 0.70 mmol/l (17%) ($p < 0.05$) (Fig 2).

Plasma HDL cholesterol levels showed a transient increase during training (Fig 1 and 2). The group mean values of all subjects together rose by 0.06 mmol/l (5%) after four weeks and by 0.08 mmol/l (7%) after six weeks ($p < 0.05$). The increase was most marked in group B (Fig 2). In this group HDL cholesterol concentrations were 0.12 mmol/l (11%) above pretraining levels after four weeks and 0.15 mmol/l (14%) ($p < 0.05$) after six weeks, compared to an increase of only 0.04 mmol/l (3%) after four and six weeks of training in group A (Fig 1). With continued training the HDL cholesterol levels fell again, and were only 0.04 mmol/l (4%) (n.s.) above the initial levels in group B after 12 weeks (Fig 2).

In subjects from group A and B, who were studied 6 weeks after the end of the training program, plasma concentrations of lipids and lipoproteins had returned to the initial levels again.

The protein moiety of HDL, monitored by measurements of apolipoprotein AI, increased by about 7% ($p < 0.01$) after four and six weeks in both groups (Fig 1, 2), but decreased again after continued training (Fig 2).

In a subsample of eight subjects in group A the time-course of alterations of the other HDL constituents was further studied. After six weeks the concentrations of free cholesterol, cholesteryl ester and phospholipid were 0.02 mmol/l (10%), 0.06 mmol/l (6%) ($p < 0.05$) and 0.10 mmol/l (8%) above initial levels, while the minor

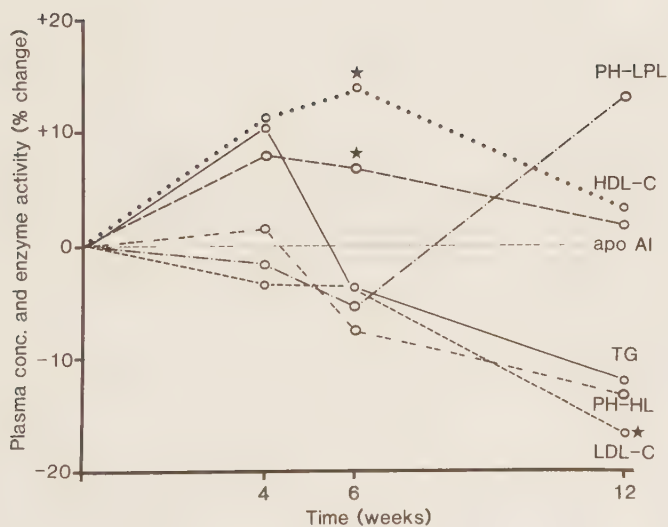


Fig 2. Relative changes in plasma lipoprotein and apo AI concentrations and heparin releasable lipolytic enzyme activities in 6 subjects (group B) during 12 weeks of training. Initial values were (mean $\pm$ S.D.): Triglycerides 1.2 ± 0.7 mmol/l, LDL cholesterol 3.9 ± 0.7 mmol/l, HDL cholesterol 1.08 ± 0.20 mmol/l, apo AI $98 \pm 2\%$. LPL 1.69 ± 0.65 μ kat/l and HL 7.58 ± 2.20 μ kat/l. * = < 0.05 , ** = $p < 0.01$. Abbreviations as in Fig 1.

triglyceride fraction decreased by 0.05 mmol/l (20%) ($p < 0.05$) (Fig 3).

As a consequence of the differential changes in HDL components the lipid composition of the HDL particle changed after training (Table 2). The proportions of the free and esterified cholesterol fraction remained unchanged. The surface component, phospholipid, expanded ($p < 0.05$) at the expense of the minor core component the triglyceride, which occupied 3% less of the lipid moiety of HDL after training.

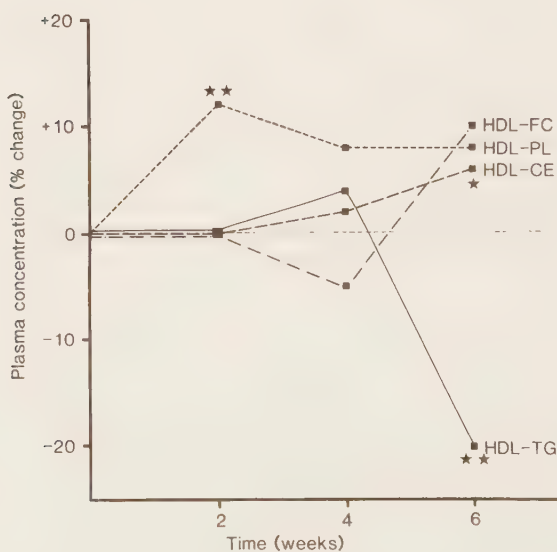


Fig 3. Relative changes in plasma concentrations of HDL components during 6 weeks of training in 8 subjects (group A). Initial values were (mean $\pm$ S.D., mmol/l): HDL free cholesterol 0.20 ± 0.07 , HDL cholesteryl ester 1.02 ± 0.26 , HDL phospholipid 1.23 ± 0.18 and HDL triglyceride 0.24 ± 0.09 . * = $p < 0.05$, ** = $p < 0.01$.

TABLE 2 Lipid composition of HDL before and after 6 weeks of training in eight subjects.

	Total chol	Chol ester	Free chol	Phospho- lipid	TG
Before	38.1 ⁺ _{2.1}	34.2 _{1.8}	3.9 _{0.3}	49.9 _{0.7}	12.0 _{1.9}
After 6 w	38.5 _{2.3}	34.5 _{1.9}	4.0 _{0.5}	52.5 _{1.1} *	9.0 _{1.4} **

(%, w/v, mean ₋ SEM)

* = $p < 0.05$

** = $p < 0.01$

In addition, we separated lipoproteins from four of these subjects by zonal ultracentrifugation to quantitate and characterize the lipoprotein classes and HDL subclasses in order to reveal changes in the HDL₂/HDL₃ ratio and the composition of the lipoprotein classes. The concentration of LDL remained constant while VLDL and HDL₃ concentrations increased moderately (Fig 4). The most marked increase, 36%, occurred in the HDL₂ subclass, leading to an elevation of the HDL₂/HDL₃ ratio by 18% (Fig 4).

The changes in composition of the lipoprotein classes are shown in table 3. The composition of LDL did not change. The protein moiety of all other classes expanded at the expense of the core components.

The expansion of the protein constituent was most marked in the HDL₂ subclass where it occurred mainly at the expense of the cholesteryl ester fraction. In VLDL the expansion of the protein moiety occurred at the expense

TABLE 3 Composition of lipoprotein classes separated by zonal ultracentrifugation in four subjects before and after six weeks of training

		TG (%)	Free chol (%)	Chol ester (%)	Phospho-lipid (%)	Protein (%)
VLDL	before	46.9	5.7	17.4	18.0	11.9
	after	44.8	7.0	15.7	18.2	14.3
LDL	before	7.5	7.8	41.0	21.4	22.3
	after	7.9	8.6	39.2	22.0	22.2
HDL ₂	before	7.2	4.3	21.6	25.1	41.9
	after	7.2	3.8	17.0	25.0	47.0
HDL ₃	before	4.3	2.1	16.4	21.4	55.8
	after	3.7	1.8	15.6	20.4	58.4

of another core component, the triglyceride fraction.

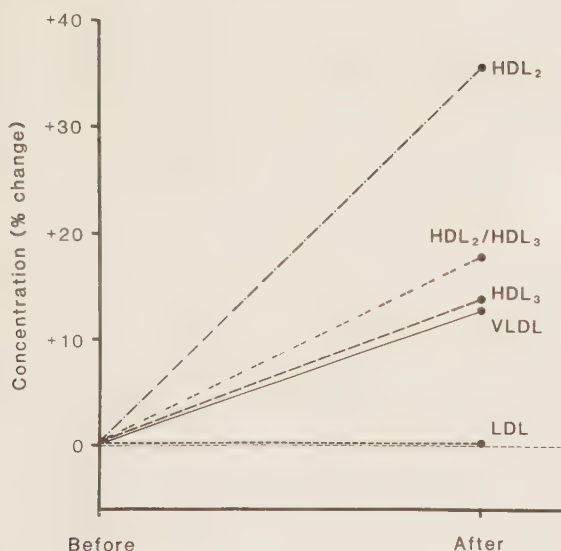


Fig 4. Relative changes in the mean concentrations of lipoprotein classes separated by zonal ultracentrifugation in four subjects before and after six weeks of training.

These changes indicate that the size of both VLDL and HDL₂ particles decrease during training.

Lipoprotein lipase and hepatic lipase

Although a tendency towards increasing values, + 13% (n.s.), was seen in group B after twelve weeks of training (Fig 2), lipoprotein lipase activity in postheparin plasma remained essentially unchanged in both groups (Fig 1, 2). The activity of hepatic lipase, however, de-

TABLE 4 Lipoprotein lipase activities in group A before and after six weeks of training (mean $\pm$ SEM)

	Before	After	Mean $\pm$ 2SD of control group
Postheparin plasma LPL activity (μ kat/l), n 12	1.69 $\pm$ 0.65	1.69 $\pm$ 0.18	1.17 - 2.42
Skeletal muscle LPL activity (pkat/g wet weight) n=11	32.9 $\pm$ 6.7	31.1 $\pm$ 5.9	-
Adipose tissue LPL activity (pkat/g lipid) n=12	101.0 $\pm$ 23.3	153.0 $\pm$ 46.9*	86.8-247.2

* p < 0.05

creased to about the same extent in both groups (Fig 1,2) and was in all eighteen subjects together 6% ($p < 0.05$) below initial values after six weeks.

The lipoprotein lipase activity of skeletal muscle did not change, but that of adipose tissue showed a marked increase of about 50% ($p < 0.05$) at the end of the training period (Table 4).

Correlations

There was no correlation between initial HDL levels and change in HDL concentrations. Correlation coefficients between HDL cholesterol, apolipoprotein AI and variables reflecting body composition and lipolytic enzyme activities are shown in table 5.

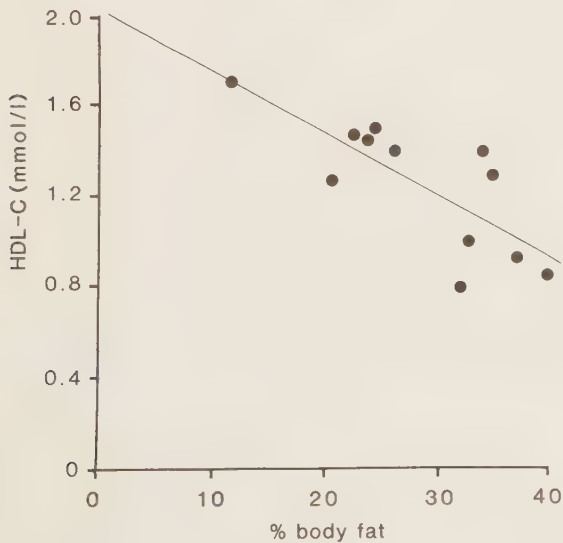


Fig 5. Linear regression of per cent body fat and HDL cholesterol concentrations before training. Regression equation, $y = - 0.03X + 2.03$; correlation coefficient, $- 0.77$ ($p < 0.01$), $n = 12$.

TABLE 5 Correlation coefficients between HDL concentrations, body weight and body composition and lipolytic enzyme activities before training

	HDL chol	apo AI	number of subjects
Broca's index	- 0.68**	- 0.45*	18
Per cent body fat	- 0.77**	- 0.45	12
Postheparin HL activity	- 0.41*	- 0.69**	18
Postheparin LPL activity	0.26	0.43*	18
Adipose tissue LPL activity	0.29	0.55*	12
Skeletal muscle LPL activity	0.33	0.63*	11

* $p < 0.05$

** $p < 0.01$

Before training Broca's index (Table 5) and per cent body fat (Fig 5) showed significant negative correlations with HDL cholesterol levels. The change in per cent body fat was negatively correlated to the change in HDL cholesterol and apo AI, correlation coefficients, -0.48 (n.s.) and -0.52 ($p < 0.05$), respectively. Changes in HDL cholesterol were also correlated with changes in fat ingestion (Fig 6).

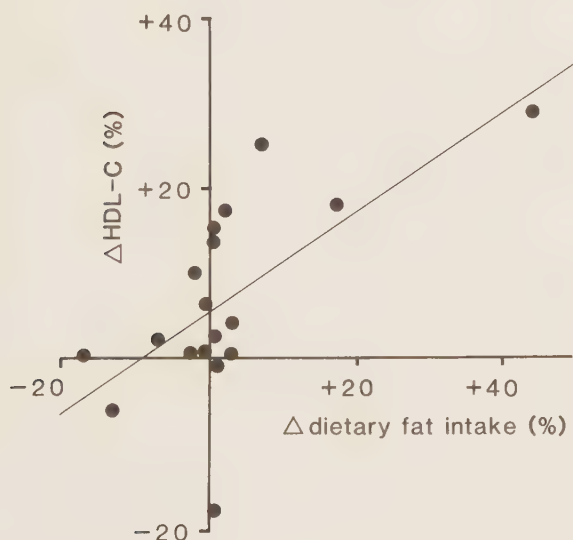


Fig 6. Linear regression of relative changes in fat ingestion and HDL cholesterol concentrations after six weeks of training. Regression equation, $y = 0.59X + 5.48$; correlation coefficient, 0.65 ($P < 0.01$), $n = 18$.

There were no significant relationships between initial VO_2 max and plasma lipoprotein concentrations or between the change in these variables. The frequency of weekly

training sessions and total energy expenditure during the program was not correlated to the changes in HDL levels. Rather, there seemed to be a negative correlation between the increase in HDL cholesterol concentrations and training intensity (Fig 7).

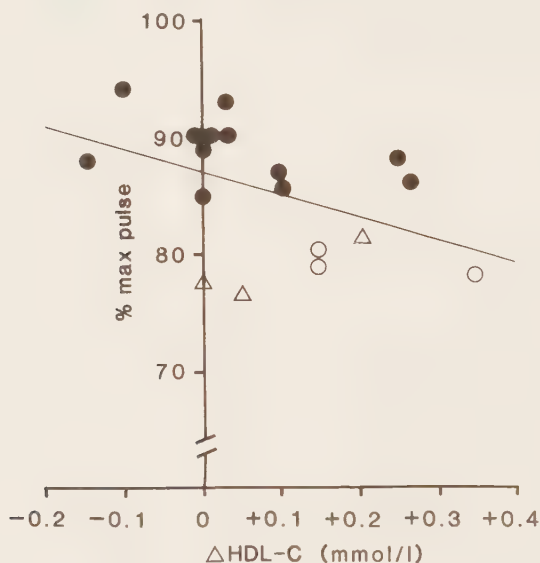


Fig 7. Linear regression of training intensity (training pulse rate expressed as per cent of maximal pulse rate) and changes in HDL cholesterol concentrations after six weeks of training. Regression equation, $y = -19.07X + 87.01$; correlation coefficient, -0.47 ($p < 0.05$) $n = 18$. ● = group "A", ○ = group "B" training three times a week, Δ = group "B" five times a week.

Changes in VO_2 max were significantly correlated with changes in LPL activity in postheparin plasma, adipose tissue, and skeletal muscle with correlation coefficients of 0.51, 0.55 and 0.51 ($p < 0.05$) respectively.

Before training, HDL cholesterol and apo AI levels were correlated with HL activities; apo AI levels were further correlated to LPL activities (Table 5). There was also a significant correlation between changes in postheparin plasma LPL activity and HDL cholesterol concentrations in group B after twelve weeks of training, correlation coefficient, 0.77 ($p < 0.05$).

DISCUSSION

During the training program all lipoprotein fractions changed in a time dependent manner. The transient, initial increase in plasma triglyceride concentrations (in these fasting subjects essentially reflecting VLDL concentrations), has been noted previously (9, 13, 30, 31), but rarely commented upon. Increased energy intake (13) or increased fatty acid mobilization from adipose tissue (32) leading to enhanced VLDL synthesis during the initial adjustment to exercise could explain this finding.

The decrease in LDL is a well-known and consistent finding in training studies (8). The reduction seems to be related to the amount of exercise rather than to the intensity and although the mechanisms remain obscure, enhanced LDL catabolism has been suggested (11).

The increase in HDL cholesterol and apo AI concentrations was of about the same magnitude and showed a similar time-course as earlier reported (7, 9, 10, 33), even if HDL and apo AI levels fell again with continued training. Also in this study (cf 7, 9, 10, 33) there was a lack of relationship between initial physical fitness and HDL

concentrations.

The difference in HDL increase between group 'A' and 'B' could not be ascribed to differences in initial HDL concentrations. It was not related to differences in the frequency of training sessions, to total energy expenditure or to the change in physical fitness. This indicates that training intensity as such might be of importance (34), especially since we found a significant negative correlation between change in HDL cholesterol and training intensity (expressed as percent max pulse). Some of the variability of the results in different longitudinal training studies might also be due to differences in the intensity of the program. However, this relationship needs to be clarified in future studies. Since it was comparatively weak, other factors are probably more important for the rise in HDL.

Weight loss is one of the factors known to increase HDL cholesterol and apo AI levels (35), but in this study the negligible change in mean total body weight and the lack of correlation between these variables indicated that the alterations in lipoprotein profile observed is not explained by weight reduction as such. Measurements of body fat seem necessary to detect an association between plasma lipoprotein concentrations and metabolic compartments. Some authors have demonstrated highly significant correlations between the increase in HDL cholesterol concentrations and the relative decrease in body fat (35, 36). Although we found only minimal changes in body fat we were able to demonstrate significant correlations between the latter variable and changes in apo AI and HDL cholesterol levels. This finding suggests that a reduction in body fat might contribute to the

rise in HDL concentrations during exercise programs.

Moreover, increases in energy and fat ingestion have been shown to influence HDL concentrations (37). Also in the present study such a relationship was found suggesting that alterations in diet during training might affect the lipoprotein profile. Alcohol intake and cigarette smoking, which also markedly affect HDL levels (37, 38), remained constant.

The exact mechanisms whereby physical exercise leads to higher HDL concentrations remain speculative, but increasing evidence supports the view that the activity of LPL and HL is of major importance. LPL catalyzes the degradation of VLDL and chylomicrons to LDL, a process associated with an elevation of HDL levels, whereas HL is believed to be involved in the degradation of HDL particles in the liver (39). Several cross-sectional studies have demonstrated lower HL activities in post heparin plasma and higher LPL levels in skeletal muscle and adipose tissue from physically active subjects (40, 41).

Our data, demonstrating an increase in adipose tissue LPL and a decrease in HL activities during training, are consistent with the study of Peltonen (33) and support present views on the physiological roles of the post heparin lipases (39). The mean post heparin plasma LPL activity did not increase significantly (cf 33), but the role of LPL in HDL regulation is demonstrated by the significant correlations between these variables.

The increase in HDL was reflected in all its major components. The protein moiety of HDL, monitored by measu-

rements of apolipoprotein AI, increased in parallel with HDL cholesterol levels and to about the same extent as previously reported (10). Among the other components of HDL, the phospholipid fraction increased most rapidly, whereas changes in free cholesterol, cholesteryl ester and triglyceride did not occur until after 4 - 6 weeks of training. Except for the minor triglyceride component, which decreased by 20%, all constituents of HDL increased by about 6 - 12%. The differential effects on the HDL components led to a significant change in the lipid composition of HDL during training (Table 3) with an increase of the surface component phospholipid and a reduction of the core component, the triglyceride.

Such differences in total HDL composition may be due to qualitative changes in HDL composition, to quantitative changes in the proportions of the HDL₂ and HDL₃ subfractions, or to a combination of these possibilities. Significantly higher HDL₂ concentrations have been demonstrated in runners compared to controls (42). In accordance with an earlier study (13) the training program led to an increase in the HDL₂ subfraction. We also noted changes in the composition of the lipoprotein classes (Table 3), generally representing increases of surface components at the expense of core components. The quantitative changes of HDL subclasses and the qualitative changes within each HDL subclass are consistent with the changes noted in the lipid composition of the HDL supernatant (Table 2). Our data on HDL subfractions differ from the results of Lipson (12), who could not demonstrate changes in HDL and its subfractions during training. Possibly, this discrepancy could be due to the fact that his subjects were kept on a iso-weight diet.

In conclusion, the present study shows that physical training leads to an elevation of plasma HDL levels, concomitant with moderate changes in HDL composition and an increase of the $\text{HDL}_2/\text{HDL}_3$ ratio. According to present views, these alterations, seem beneficial from the atherogenic point of view (43). The increase in HDL levels is most prominent during the initial adaptation to exercise but tends to dampen out with continued training. Our study suggests that factors accompanying the institution of the training program, such as changes in body composition and diet may contribute to the elevation of HDL besides the training as such. Also, our data indicate that the effects of training on HDL are partly mediated through effects on HL and LPL activities.

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High-density lipoprotein concentrations increase after stopping smoking

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PETER NILSSON-EHLE

Abstract

Concentrations of plasma lipoproteins in 10 men who were habitual smokers were monitored for six weeks after they stopped smoking and related to changes in diet and body weight. The energy intake increased by 10% ($p < 0.05$) owing to a higher consumption of carbohydrate and fat, and body weight increased by 2% ($p < 0.01$). Plasma triglyceride, cholesterol, and low-density lipoprotein cholesterol concentrations did not change significantly.

The most prominent finding was a rapid and pronounced increase in high-density lipoprotein concentrations. From comparatively low values (mean 0.82 mmol/l) they rose by 29% ($p < 0.01$) within two weeks and remained at this value throughout the observation period. In three subjects who resumed smoking after the end of the study they again fell to initial values six weeks later. The initial increase in concentration could be accounted for mainly by an increase in the esterified fraction and only to a lesser extent in the free cholesterol fraction. The changes in concentration were accompanied by similar but less pronounced rises in high-density lipoprotein phospholipid and in apolipoprotein AI concentrations ($p < 0.01$), whereas high-density lipoprotein triglyceride concentrations did not change significantly.

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These findings confirm and extend those of earlier cross-sectional studies which showed low concentrations of high-density lipoproteins in cigarette smokers. A significant correlation between the rise in high-density lipoprotein cholesterol concentrations and the increase in fat consumption after stopping smoking indicate that the changes in high-density lipoprotein concentrations may be partly due to nutritional factors.

Introduction

The association between cigarette smoking and coronary heart disease is well established,¹⁻⁴ but the mechanisms by which smoking harms the heart are poorly understood.^{5,6} One of the factors which may increase the risk of coronary heart disease is the low concentration of high-density lipoprotein found in cigarette smokers.⁷⁻⁹

To assess further the relationship between smoking and plasma lipoprotein concentrations we have monitored the time-course for changes in lipoprotein concentrations after subjects stop smoking completely and related these to changes in diet and body weight.

Subjects and methods

Twenty-one male smokers, recruited by advertising in the local hospital bulletin, were included in the study and 10 of them succeeded in abstaining from smoking during the observation period of six weeks. Their age range was 32-50 (mean 38) years and weight 64-100 (mean 77) kg. All had been heavy smokers for at least 12 years with a consumption of 17-30 (mean 21) cigarettes a day. They had normal Swedish dietary habits, and none was engaged in regular physical activities. All felt well, and none took any medication. Routine laboratory tests showed no signs of renal, hepatic, or metabolic disorders.

While abstaining from smoking the subjects were supported psychologically by a team from the hospital's smoking withdrawal clinic, but no medication was given. Dietary habits were registered by a clinical nutritionist, who obtained a careful dietary history both during and at the end of the study.¹⁰ The food composition tables edited by the Swedish National Food Administration were used to calculate the proportions of fat, protein, and carbohydrates.

Blood samples taken after one, two, four, and six weeks were collected in the morning, after the subjects had been fasting for at least 10 hours. The initial values represented the mean of two samples drawn at one week and at three days before stopping smoking.

The lipid components of high-density lipoprotein were determined in the supernatant obtained after precipitation of very low-density lipoprotein and low-density lipoprotein by magnesium chloride and dextran sulphate.¹¹ Cholesterol and triglycerides were determined by enzymatic methods¹¹⁻¹³; free and esterified cholesterol were differentiated by measurements with and without the cholesterol esterase reagent. Phospholipids were measured as lipid phosphorus. Apolipoprotein AI was measured in plasma by electroimmunoassay.¹⁴ Low-density lipoprotein cholesterol was calculated according to the formula of Friedewald.¹⁵

The significance of changes in different variables was evaluated using Wilcoxon's rank order test for paired observations. Correlations between variables were analysed by linear regression analysis.

Results

Carboxyhaemoglobin concentrations were raised before stopping smoking ($3.4 \pm 1.0\%$, mean $\pm$ SD) and fell to below 1% within one week after stopping smoking. The initial concentrations of carboxyhaemoglobin were correlated with the number of cigarettes smoked ($r=0.68$; $p<0.05$), but there was no correlation between these two variables and the initial concentrations of high-density lipoprotein cholesterol or apolipoprotein AI.

A small transient decrease of about 5% occurred in haemoglobin concentrations, red blood cell count, and packed cell volume during the first two weeks ($p<0.01$), but all values were in the upper part of the reference range (initial mean haemoglobin concentration was 14.4 g/dl).

The concentrations of plasma proteins, albumin, α_1 -antitrypsin, orosomucoid, haptoglobin, ceruloplasmin, and IgG did not change, but IgA concentrations decreased by about 20% ($p<0.01$).

The initial mean energy intake was 11 676 (range 7896-18 564) kJ per day consisting of 16% protein, 39% fat, 42% carbohydrates, and 3% alcohol. After stopping smoking the mean energy intake increased by 9% after three weeks ($p<0.05$) and was about 7% above initial values at the end of the study (not significant). This increase could be accounted for by a higher consumption of carbohydrates and fat (+10% and +9%, $p<0.05$ after three weeks). Alcohol intake did not change in the middle of the observation period and decreased slightly by about 9% (not significant) at the end of the study. Body weight increased by a mean of 1.8 kg or 2% ($p<0.01$). The physical activity of the subjects, assessed by detailed interview, did not change during the study.

The most prominent change in the plasma lipid and lipoprotein concentrations was a rapid and pronounced rise in high-density lipoprotein concentrations after stopping smoking. From comparatively low values (mean 0.82 mmol/l, lower reference limit 0.8 mmol/l) concentrations gradually increased and reached a plateau 29% above initial values ($p<0.01$) after two weeks (fig 1). Individual

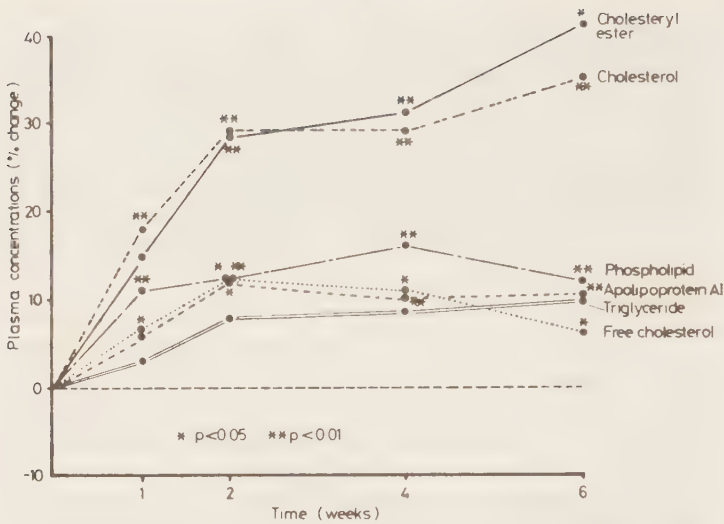


FIG 1—Relative changes in high-density lipoprotein (HDL) constituents after stopping smoking. Initial values (mmol/l, mean $\pm$ SEM) were: cholesteryl ester, 0.61 ± 0.06 , free cholesterol 0.21 ± 0.01 , total cholesterol, 0.82 ± 0.04 , phospholipid, 0.95 ± 0.04 , triglyceride, 0.20 ± 0.01 , and apolipoprotein AI (% mean $\pm$ SEM), 94 ± 4 .

initial and maximum concentrations are shown in table I. The rise in high-density lipoprotein cholesterol concentrations was associated with a transient moderate increase in plasma cholesterol concentrations of about 7% ($p < 0.01$) during the first two weeks. Low-density lipoprotein cholesterol concentrations remained unchanged. Plasma triglyceride concentrations, essentially reflecting very-low-density lipoprotein concentrations in these normolipaeic subjects, were constant up to four weeks after stopping smoking but increased by about 21% ($p < 0.05$) after six weeks.

TABLE I—Increase in high-density lipoprotein (HDL) cholesterol concentrations after stopping smoking in 10 subjects

Case No	HDL cholesterol concentrations (mmol/l)		Time of maximum change (weeks)
	Initial	Maximum	
1	0.85	1.10	6
2	0.65	1.10	6
3	0.80	1.10	6
4	0.70	1.40	6
5	0.85	1.10	4
6	0.75	1.30	6
7	1.05	1.20	6
8	0.90	1.20	2
9	0.75	0.90	4
10	0.85	1.40	6
Mean ($\pm$ SEM)	0.82 (0.036)	1.18 (0.049)	

Three of the subjects resumed smoking after six weeks of abstinence. Their initial high-density lipoprotein cholesterol concentrations were 0.75-0.92 (mean 0.82) mmol/l and increased by about 13%, 24%, 22%, and 33% after one, two, four, and six weeks respectively. Four weeks after they resumed smoking the concentrations had again decreased and were only 8% above the initial values.

The rise in high-density lipoprotein was reflected in all its major components, which increased simultaneously but to varying degrees during the first two weeks after stopping smoking (fig 1). The increase in high-density lipoprotein cholesterol was mainly due to an increase in the esterified cholesterol fraction, about 41% at the end of the study ($p < 0.05$), whereas the free cholesterol fraction increased by only 10% ($p < 0.05$), a rise similar to that for high-density lipoprotein phospholipids ($p < 0.01$). The only lipid constituent of high-density lipoprotein that did not change significantly was the triglyceride component. The protein moiety of high-density lipoprotein, monitored by concentrations of apolipoprotein AI, increased by about 10% ($p < 0.01$).

As a result of the differential changes in high-density lipoprotein components, the composition of the high-density lipoprotein particles changed significantly after stopping smoking. The ratio of apolipoprotein AI to high-density lipoprotein cholesterol fell by 11% ($p < 0.05$) after two weeks, suggesting a preferential increase in the lipid-rich high-density lipoproteins particles. A comparison of the lipid composition of high-density lipoprotein before and after stopping smoking is given in table II. Most prominently, the cholesteryl

TABLE II—Percentage lipid composition of high-density lipoprotein before and six weeks after stopping smoking in 10 subjects (w/v ; means $\pm$ SEM)

	Total cholesterol	Cholesteryl ester	Free cholesterol	Phospholipid	Plasma triglyceride
Before	33.6 $\pm$ 1.2	27.5 $\pm$ 1.4	6.1 $\pm$ 0.4	53.6 $\pm$ 0.8	12.8 $\pm$ 0.6
After six weeks	38.6 $\pm$ 1.8*	32.8 $\pm$ 1.9*	5.8 $\pm$ 0.3	50.8 $\pm$ 1.4	10.6 $\pm$ 0.6

* $p < 0.05$.

ester fraction increased by 13-19% ($p < 0.05$) and constituted significantly more (5.3%) of the lipid moiety of high-density lipoprotein. This expansion occurred at the expense of all other lipid components but mainly resulted in a reduction of the proportion of the other core component, the triglyceride.

The nature of the increase in high-density lipoprotein was further investigated by statistical analysis of the relationship between high-density lipoprotein components and other lipids and lipoproteins as well as dietary constituents; both absolute values of these variables and changes occurring after stopping smoking have been analysed

by linear regression. No significant relationships were found between high-density lipoprotein concentrations and other plasma lipids and lipoproteins. There were no significant correlations between changes in carboxyhaemoglobin concentrations, body weight, or carbohydrate or alcohol consumption on the one hand and high-density lipoprotein concentrations on the other. The increase in fat ingestion was, however, significantly correlated with the rise in high-density lipoprotein cholesterol ($r = 0.73$, $p < 0.01$) (fig 2).

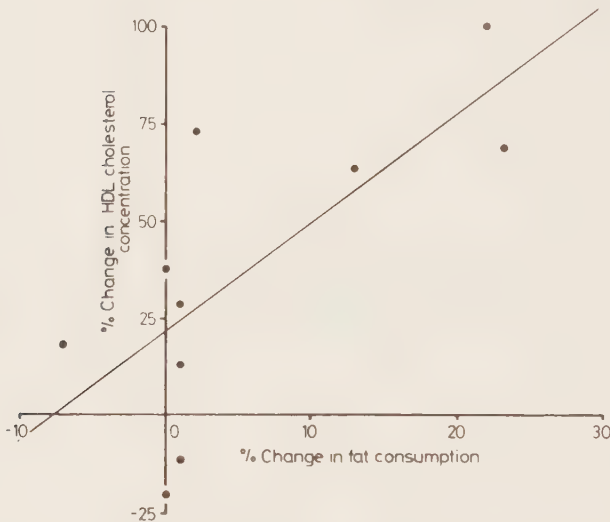


FIG 2—Linear regression of relative changes in dietary fat intake and high-density lipoprotein (HDL) cholesterol concentrations. Regression equation, $y = 2.8 + 21.8x$; correlation coefficient 0.73 ($p < 0.01$).

Discussion

Cigarette smoking is one of the major risk factors in coronary heart disease.¹⁻⁴ Several mechanisms have been suggested to explain the harmful effects of cigarette smoking on cardiovascular health⁵⁻⁶; several recent studies have focused on changes in the lipoprotein profile as possible reasons. In several comparative studies of smokers and non-smokers, smokers have had significantly lower high-density lipoprotein concentrations, measured as high-density lipoprotein cholesterol⁷⁻⁹ or as apolipoprotein AI¹⁰; in some studies, moderately raised low-density lipoprotein concentrations have also been found.¹⁷⁻¹⁸ To clarify further the relation between smoking and changes in

lipoprotein values, we have monitored the time-course of changes in lipoprotein concentrations, particularly those of high-density lipoprotein and its constituents, in 10 heavy smokers who stopped smoking completely.

In agreement with earlier findings, our subjects had remarkably low concentrations of high-density lipoprotein cholesterol and apolipoprotein AI before stopping smoking. In fact, five out of the 10 participants had high-density lipoprotein cholesterol concentrations below the lower reference limit (0.8 mmol/l). With the limited number of subjects, no significant relation between carboxyhaemoglobin concentrations or cigarette consumption and high-density lipoprotein concentrations could be shown.⁸⁻⁹ The initial low-density lipoprotein concentrations recorded in our study were not higher than normal.¹⁷⁻¹⁸

The participants in the study were encouraged to keep their habits and physical activities as constant as possible after stopping smoking. Nevertheless, almost all increased their consumption of fat and carbohydrate by about 7-10%, but the weight gain (mean 1.8 kg over six weeks) was less pronounced than usually recorded.¹⁹ Alcohol intake, which rapidly raises high-density lipoprotein concentrations,²⁰ fell towards the end of the study.

Except for the minor triglyceride component, all constituents of the high-density lipoprotein fraction increased significantly by 10-15% for apolipoprotein AI, phospholipid, and free cholesterol. Nevertheless, the major component, cholesteryl ester, increased by more than 40%. The corresponding changes in high-density lipoprotein composition suggest that a shift in the ratio of high-density lipoprotein₂ to high-density lipoprotein₃ occurred, with a more pronounced increase in the lipid-rich high-density lipoprotein₂ particles.

The close relation between smoking and high-density lipoprotein concentrations is further illustrated by the fact that in those subjects who resumed smoking after the end of the study concentrations rapidly returned to the low initial values. The mechanisms responsible for the pronounced increase in high-density lipoprotein concentrations remain unclear. Nevertheless, the correlation between the increase in fat intake and rise in high-density lipoprotein cholesterol concentrations indicates that the changes in lipoprotein concentrations after stopping smoking may be partly related to nutritional changes.

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V.

ALTERATIONS IN HDL SUBFRACTIONS AND COMPOSITION AFTER
SMOKING CESSATION: RELATIONSHIPS TO LIPOPROTEIN LIPASE,
HEPATIC LIPASE AND LCAT ACTIVITIES

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Submitted for publication

ABSTRACT

Alterations in the plasma levels of lipoproteins, including high density lipoprotein (HDL) subfractions and components, were followed in nineteen healthy male cigarette smokers during four weeks complete abstinence from smoking and related to changes in the activities of lipoprotein lipase, hepatic lipase and lecithin: cholesterol acyltransferase. The subjects increased their intake of dietary fat and carbohydrates by about 10% ($p < 0.01$) and a weight-gain of 2% ($p < 0.01$) was observed. During the latter part of the study a moderate decrease in low-density lipoprotein cholesterol (LDL) (8%, $p < 0.01$) was noted while plasma cholesterol and triglyceride levels remained constant.

HDL cholesterol levels increased rapidly to levels 18% ($p < 0.01$) above the initial, after two weeks study. Similar increases were seen in the free and the esterified fraction of HDL cholesterol and in apolipoprotein AI. The rise in HDL phospholipid did not reach statistical significance and HDL triglyceride remained unchanged. Separation of lipoproteins with zonal ultracentrifugation in nine subjects demonstrated increases in both HDL subclasses, most prominently in the HDL₂ subfraction, which rose by 32% ($p < 0.05$). VLDL and LDL remained essentially unchanged. The composition of LDL and HDL₃ remained constant, while the protein moiety of VLDL and HDL₂ increased at the expense of the triglyceride component.

Postheparin lipoprotein lipase (LPL) activities showed a transient, significant increase, whereas hepatic lipase (HL) activities remained unchanged. Also, LCAT activities rose significantly. These changes, supported by significant correlations between LPL, LCAT and HDL and its components, indicate that the increase in HDL is at least partly mediated via these key enzymes in lipoprotein metabolism. Furthermore, a highly significant correlation between changes in fat intake and HDL cholesterol levels indicated that nutritional factors contribute to the rise in HDL after smoking cessation.

INTRODUCTION

For about two decades it has been obvious that cigarette smokers have a highly increased risk for coronary heart disease (1, 2). Nevertheless, the mechanisms behind the increased cardiovascular risk largely remain unclear. Recently, much interest has focused on the lowered levels of high density lipoprotein (HDL) found in cigarette smokers compared to non-smokers (3, 4, 5). After smoking cessation, HDL concentrations show a rapid and marked increase, which partly may be due to changes in dietary habits (6).

The aim of the present study was to further characterize the rise in HDL after smoking cessation by monitoring the changes in individual HDL components and in the HDL subfractions, HDL₂ and HDL₃. Simultaneous determinations of the activities of the key enzymes in HDL regulation, lipoprotein lipase (LPL), hepatic lipase (HL) and lecithin: cholesterol acyltransferase (LCAT), were performed to elucidate the mechanisms behind the lipoprotein alterations after smoking withdrawal.

MATERIALS AND METHODS

Subjects

Thirty-six healthy, male smokers, recruited by advertising in the local hospital bulletin, were included in the study. Nineteen of them abstained completely from smoking during the observation period of four weeks. Their mean age was 38 years (range 30 - 60) and their mean body weight was 77 kg (range 64 - 100). All were heavy cigarette smokers, smoking on the average 23 cigarettes (range 17 - 35) per day since at least 12 years. They had normal Swedish diet and drinking habits and did not take part in regular sports activities. None of the subjects had laboratory signs of renal, hepatic or metabolic disease. No medication was given or allowed throughout the observation period.

Diet

Dietary habits were recorded by a clinical nutritionist who obtained a careful 'diet history' according to Burke (7, 8) before and after the study. The energy content and the composition of the food was calculated from the Food Tables edited by the Swedish National Food Administration.

Blood sampling

Blood samples were drawn between 8 and 9 a.m. after the subjects had been fasting for at least 10 h. The initial values represent the mean of two samples drawn within one week before the start of the experiment. Samples were then obtained two and four weeks after the subjects stopped smoking.

Laboratory analyses

The lipid components of HDL were determined in the supernatant obtained after precipitation of VLDL and LDL by MgCl_2 and dextran sulphate (9). Cholesterol and triglycerides were determined by enzymatic methods (10, 11); free and esterified cholesterol were differentiated by performing the measurement with and without the cholesterol esterase reagent. Phospholipids were measured by phosphorus determination in lipid extracts (12).

Apolipoprotein AI was measured in plasma by electroimmunoassay (9). Data for the apolipoproteins are expressed as per cent of the value of a plasma pool obtained from 40 healthy males. LDL cholesterol was calculated according to the formula of Friedewald (13).

Lipoprotein lipase (LPL) and hepatic lipase (HL) activities were determined in post heparin plasma drawn 15 min after the injection of 50 U heparin per kg body weight with methods described earlier (14).

Separation of plasma lipoproteins into lipoprotein classes and HDL into subfractions with zonal ultracentrifugation was performed in a subsample of nine subjects before and four weeks after smoking cessation by a modification of the technique described earlier (9). The sample (15 g EDTA plasma) was adjusted to a density of 1.295 kg/l by addition of solid NaBr, and layered under a linear NaBr gradient with a density of 1.00 - 1.30 kg/l in a Beckman Ti-14 zonal rotor. VLDL and LDL were floated by centrifugation in a Beckman L-2-65B ultracentrifuge at 44.000 rpm for 2 h. The rotor was then decelerated to 3.000 rpm, and about 350 ml of the rotor content (containing VLDL and LDL) was evacuated. The gradient was then reconstructed, and the HDL subfractions separated by further centrifugation at 44.000 rpm for 22 h after which fractions were collected as above. The protein content of the collected fractions was continuously monitored by spectrophotometry at an absorbance of 280 nm. The lipoprotein classes were quantitated by planimetry of the respective absorbance peaks. The composition of the lipoprotein classes was determined in representative pools of each peak by the methods described above. Protein was measured by a scaled-down version of Lowry's method (15).

In the same subjects the endogenous cholesterol esterifying ability was determined according to Stokke and Norum (16). The cholesterol esterification rate was also measured using normal serum as substrate with the technique described by Glomset and Wright (17).

Statistical analyses

The significance of changes in different variables was evaluated using Wilcoxon's rank order test for paired observations. Correlations between variables were analyzed by linear regression analysis.

RESULTS

Carboxyhemoglobin

The initial mean carboxyhemoglobin (COHb) concentrations were elevated, 3.4 ± 1.0 % (mean $\pm$ S.D.) and fell rapidly below 1% ($p < 0.01$) after smoking cessation. Baseline COHb levels were not correlated with the number of cigarettes smoked.

Diet and body weight

The subjects average energy intake was $11\,388 \pm 3\,318$ KJ before they stopped smoking and had increased on the average by 9% ($p < 0.01$) after 4 weeks. The diet consisted of 15% protein, 38% fat, 44% carbohydrates, 3% alcohol; these proportions remained essentially unchanged. The increase in energy intake could be accounted for mainly by an increase in fat and carbohydrate intake of 9% and 12%, respectively ($p < 0.01$); their protein consumption increased by 5% ($p < 0.05$). Alcohol intake was initially 12.7 ± 11.9 g per day and increased by only 3% (n.s.). Body weight increased on the average by 1.5 kg (1.7%, $p < 0.01$); this increase was correlated to the increase in energy intake ($r = 0.63$, $p < 0.01$).

TABLE I Concentrations of plasma lipoproteins, including HDL components (mmol/l) and apolipoprotein AI (%), before and two and four weeks after smoking cessation. Data are given as mean $\pm$ S.D.

	Before	2 weeks	4 weeks
Plasma cholesterol	5.23 $\pm$ 1.02	5.33 $\pm$ 1.26	5.26 $\pm$ 1.09
Plasma triglycerides	1.28 $\pm$ 0.46	1.25 $\pm$ 0.56	1.39 $\pm$ 0.85
LDL cholesterol	3.72 $\pm$ 1.05	3.64 $\pm$ 1.26	3.42 $\pm$ 1.02**
HDL cholesterol	0.97 $\pm$ 0.26	1.14 $\pm$ 0.27**	1.12 $\pm$ 0.24**
HDL cholesteryl ester	0.77 $\pm$ 0.26	0.88 $\pm$ 0.21**	0.89 $\pm$ 0.24*
HDL free cholesterol	0.21 $\pm$ 0.05	0.24 $\pm$ 0.06*	0.23 $\pm$ 0.06*
HDL phospholipid	1.07 $\pm$ 0.20	1.15 $\pm$ 0.19	1.11 $\pm$ 0.19
HDL triglyceride	0.18 $\pm$ 0.39	0.19 $\pm$ 0.44	0.18 $\pm$ 0.40
apolipoprotein AI	100 $\pm$ 15	107 $\pm$ 15**	105 $\pm$ 15**

* = p < 0.05

** = p < 0.01

Plasma lipoproteins and apolipoprotein AI

Mean concentrations of plasma lipoproteins, including HDL components, and apolipoprotein AI before and after smoking cessation are given in Table I.

Plasma cholesterol and triglyceride levels remained essentially unchanged. LDL cholesterol concentrations were virtually constant during the first two weeks and then fell 8% below baseline values ($p < 0.01$) after 4 weeks.

Initial mean HDL cholesterol levels, in six of the subjects below 0.8 mmol/l (the lower reference limit in our laboratory), showed a rapid and marked increase (Fig 1) to levels 18% and 16% above the initial after two weeks ($p < 0.01$).

The relative changes of the various components of HDL are shown in Fig 1. Except for the minor triglyceride fraction, which remained almost unchanged, all constituents of HDL increased with similar time-courses. Apo AI levels, reflecting the protein moiety of HDL increased by 6% ($p < 0.01$) and 4% ($p < 0.05$) after two and four weeks, respectively. The rise in HDL phospholipid, the other surface component, was of about the same magnitude but did not reach statistical significance. The free and the esterified fractions of HDL cholesterol increased in parallel and to about the same extent. Two weeks after the subjects had quit smoking HDL free cholesterol and cholesteryl ester concentrations were 15% ($p < 0.05$) and 14% ($p < 0.01$), four weeks 11% and 16% ($p < 0.05$) above baseline values, respectively.

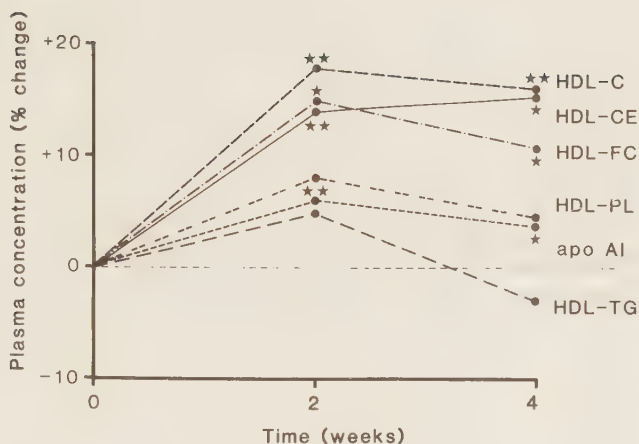


Fig 1. Relative changes in high density lipoprotein constituents after smoking cessation. Absolute initial mean values are given in Table I.

* = $p < 0.05$, ** = $p < 0.01$, $n = 19$.

As a consequence of the differential increases in HDL components the lipid composition of HDL was altered. As shown in table II, the cholesteryl ester fraction expanded mainly at the expense of the other core component, triglyceride, but also of the surface component, phospholipid. After four weeks of smoking abstinence the cholesteryl ester fraction occupied 3% more of the lipid moiety of HDL than before smoking cessation.

In order to further quantitate and characterize changes in the lipoprotein classes, including the HDL subclasses, lipoproteins were separated by zonal ultracentrifugation

TABLE II Relative lipid composition of HDL in 19 subjects before and two and four weeks after smoking cessation. Data are given as mean $\pm$ S.E.M.

	Total cholesterol	Cholesteryl ester	Free cholesterol	Phospholipid	Triglyce- ride
Before	35.9 $\pm$ 1.0	30.7 $\pm$ 1.2	5.2 $\pm$ 0.3	53.5 $\pm$ 0.6	10.6 $\pm$ 0.7
After two weeks	37.8 $\pm$ 0.6 [*]	32.5 $\pm$ 0.7	5.3 $\pm$ 0.3	52.4 $\pm$ 0.4 [*]	9.9 $\pm$ 0.6
After four weeks	38.8 $\pm$ 0.9 [*]	33.6 $\pm$ 1.0 [*]	5.2 $\pm$ 0.3	51.8 $\pm$ 0.6 [*]	9.4 $\pm$ 0.6 [*]

* = p < 0.05
** = p < 0.01

in a subsample of nine subjects before and four weeks after smoking withdrawal. The concentrations of all lipoprotein classes, as monitored by UV absorbance of the respective peaks, increased moderately; the rise in VLDL and LDL (20% and 16%, respectively) did not reach statistical significance. The most pronounced increase was seen in the HDL₂ subfraction, which rose by 32% ($p < 0.05$), twice as much as the HDL₃ subfraction, 16% ($p < 0.05$) (Fig 2). Due to the more marked elevation in HDL₂ concentration the HDL₂/HDL₃ ratio increased by 14% (Fig 2).

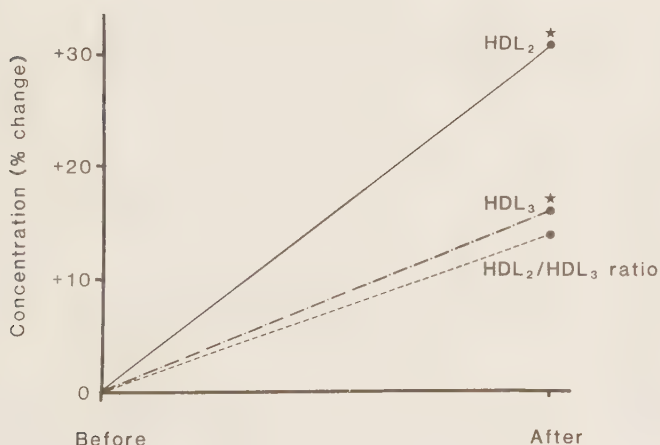


Fig 2. Relative changes of the concentrations of HDL subfractions and HDL₂/HDL₃ ratio in nine subjects before and four weeks after smoking cessation.*= $p < 0.05$.

The composition of LDL and the HDL₃ subclass remained essentially constant (Table III) while minor changes occurred in the other lipoprotein fractions.

TABLE III

Relative composition of lipoprotein classes and HDL subfractions in eight subjects before and four weeks after smoking cessation (mean $\pm$ S.E.M.)

	Triglyceride	Free Cholesterol	Cholesterol ester	Phospholipid	Protein
VLDL Before	64.8 $\pm$ 1.6	3.5 $\pm$ 0.5	5.2 $\pm$ 0.3	9.5 $\pm$ 1.3	17.0 $\pm$ 1.9
After	60.0 $\pm$ 1.4	4.0 $\pm$ 0.4	5.3 $\pm$ 1.3	6.5 $\pm$ 1.9	24.2 $\pm$ 2.0
LDL Before	5.1 $\pm$ 0.3	9.4 $\pm$ 0.2	40.4 $\pm$ 0.8	21.5 $\pm$ 0.4	23.6 $\pm$ 0.4
After	5.2 $\pm$ 0.5	8.9 $\pm$ 0.3	40.1 $\pm$ 0.6	22.0 $\pm$ 0.4	23.8 $\pm$ 0.7
HDL ₂ Before	8.1 $\pm$ 1.3	4.2 $\pm$ 0.2	17.7 $\pm$ 1.0	25.1 $\pm$ 1.3	44.9 $\pm$ 2.2
After	7.1 $\pm$ 0.8	3.9 $\pm$ 0.4	17.9 $\pm$ 1.4	24.1 $\pm$ 1.9	47.0 $\pm$ 3.1
HDL ₃ Before	2.9 $\pm$ 0.2	2.1 $\pm$ 0.1	17.6 $\pm$ 0.6	24.1 $\pm$ 0.9	53.3 $\pm$ 1.0
After	3.0 $\pm$ 0.4	2.0 $\pm$ 0.1	17.0 $\pm$ 0.7	23.2 $\pm$ 0.9	54.8 $\pm$ 1.3

The protein moiety of VLDL and HDL₂ decreased mainly at the expense of the triglyceride and phospholipid fractions (Table III).

The relative changes in lipid moiety of the HDL₂ subfraction were comparable to those seen in total HDL determined in the supernatant after precipitation of VLDL and LDL (Table II) indicating that the alterations in lipid composition of HDL occur exclusively in this subfraction. However, the compositional changes of the various lipoprotein classes separated by zonal ultracentrifugation did not reach statistical significance.

Lipoprotein lipase, hepatic lipase and LCAT activities.

Lipoprotein lipase (LPL) activity in postheparin plasma before smoking cessation was 2.23 ± 0.89 $\mu\text{kat/l}$ (mean $\pm$ SD) and had increased by 0.39 $\mu\text{kat/l}$ (18%, $p < 0.05$) after two weeks and 0.25 $\mu\text{kat/l}$ (11%, n.s.) after four weeks (Fig 3). The activity of hepatic lipase (HL) was essentially unchanged throughout the study (Fig 3).

The endogenous cholesteryl esterifying ability, determined according to Stokke and Norum, was before 5.63 ± 1.28 %/h. After two and four weeks it was 4% above initial levels, 5.84 ± 1.53 and 5.84 ± 1.35 respectively (n.s.) (Fig 3). When measured with exogenous substrate, according to Glomset and Wright, the cholesterol esterification rate demonstrated a significant increase from 2.88 ± 0.49 %/6 h, to 3.08 ± 0.62 %/6 h, after two weeks and 3.10 ± 0.53 %/6 h, 8% ($p < 0.05$) above baseline, after four weeks (Fig 3).

The cholesterol esterification rates measured with the two methods were significantly correlated (correlation coefficient, 0.71; $p < 0.05$). Also, changes in these two variables, especially during the first two weeks, were highly correlated (correlation coefficient, 0.89; $p < 0.001$).

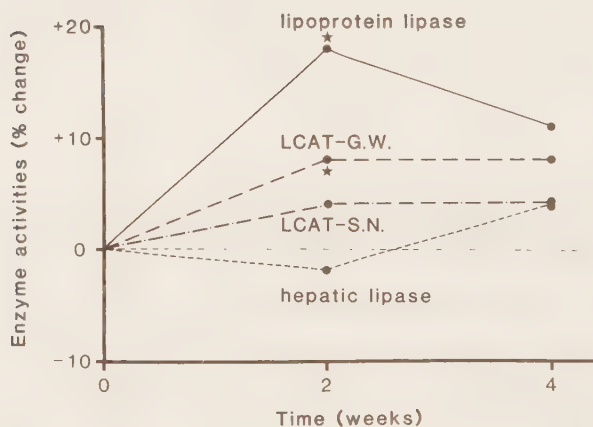


Fig 3. Relative changes in lipoprotein lipase, hepatic lipase and lecithin: cholesterol acyltransferase (LCAT) activities after smoking cessation determined according to Glomset and Wright, LCAT-G.W., and according to Stokke and Norum, LCAT-S.N. LCAT activities were determined in a subsample of nine subjects.* $p < 0.05$.

Correlations

No correlations were found between the number of cigarettes smoked, initial CHOb levels and plasma HDL concentrations nor was the decrease in COHb levels correlated to the increase in HDL cholesterol or apolipoprotein AI concentrations. No significant relationships were noted between any variable reflecting HDL concentration and changes in body weight or alcohol intake. The increase in fat consumption, however, was significantly correlated to the rise in most HDL components (Table IV), (Fig 4).

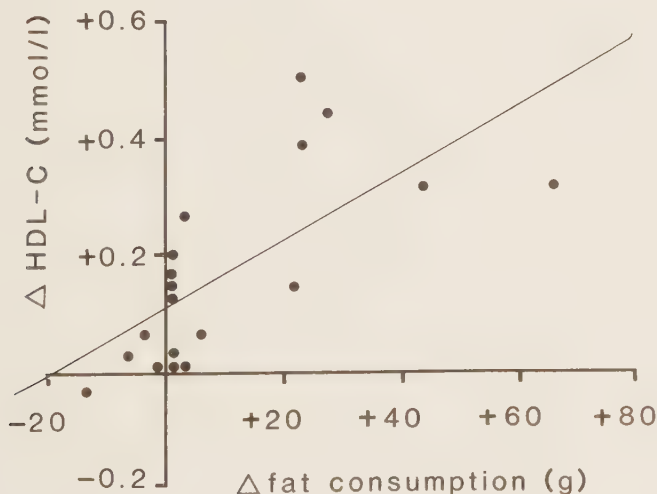


Fig 4. Linear regression of changes in dietary fat intake and HDL cholesterol concentrations.
Regression equation, $Y = 0.57x + 111.1$;
correlation coefficient, 0.69, ($p < 0.01$).

The changes in HDL components during the initial two weeks were also significantly correlated to the rise in postheparin plasma LPL activities (Table IV). No signi-

ficant correlations were found between the rise in HDL components and changes in HL activity or cholesterol esterification rate.

TABLE IV

Correlation coefficients between changes in dietary fat intake, postheparin plasma, lipoprotein lipase activities and changes in HDL concentration in nineteen subjects before and four weeks after smoking cessation. The correlation between the change in fat intake and that in LPL activity was not significant.

	Fat ingestion	Postheparin plasma LPL
HDL cholesterol	0.69 ***	0.44 *
HDL free cholesterol	0.59 **	0.42 *
HDL cholesterylester	0.57 **	0.32
HDL phospholipid	0.41 *	0.48 *
HDL triglyceride	0.29	0.41 *
Apo AI	0.55 *	0.13

* = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.001$

DISCUSSION

In large-scale comparative studies it has been repeatedly demonstrated that cigarette smokers have significantly lower levels of plasma HDL cholesterol (3, 4, 5) and apolipoprotein AI (18, 19) as well as higher LDL cholesterol concentrations (20). It seems reasonable to presume that such changes of the lipoprotein profile, which are considered unfavorable from the atherogenic point of view, may contribute to the elevated risk for CHD in smokers (1, 2).

In a recent longitudinal study we could demonstrate that HDL concentrations increase after stopping smoking (6). In the present study we have further characterized this finding and tried to elucidate the mechanisms behind the rise in HDL levels.

As expected, we found a rather small but highly significant increase in body weight after smoking cessation; the weight gain was correlated to the increase in food intake. In accordance with our earlier study, increased caloric intake was mainly accounted for by an increase in dietary carbohydrates and fat (6). There were no correlations between changes in body weight and plasma lipids and lipoproteins. Alcohol intake, which leads to an elevation of plasma HDL levels (21), did not change significantly.

Cessation of smoking led to a moderate decrease in LDL cholesterol concentrations, a finding that is consistent with the moderately increased LDL levels found in

cross-sectional studies of smokers (20). The effect of smoking cessation on LDL levels was slower than that on HDL levels and evident first after four weeks. The mechanisms for the fall in LDL levels remains speculative but might be a result of an enhanced LDL removal (22).

In accordance with our previous experience (6) and the data of comparative studies (3, 4, 5, 19), the initial HDL cholesterol levels were rather in the lower part of the reference interval. After smoking cessation, the rise in HDL cholesterol was rapid and highly significant (cf 6), suggesting that HDL levels are largely affected by smoking as such. Recently, another report of lipoprotein changes after stopping smoking has been presented (23). The changes of HDL cholesterol concentrations were strikingly similar to ours (6). In addition, data from the Multiple Risk Factor Intervention Trial (MRFIT) demonstrate substantial increases in HDL cholesterol levels in smokers who had quit smoking during the first four years of the follow-up period (24). Short-term experiments also support the view that HDL levels are affected by smoking as such (25).

The changes of the various components of the HDL particle were essentially similar to those noted before (6). In this context, it is interesting to compare our results, especially the increase in the HDL surface component, phospholipid, with data from animal experiments, showing a reduction in the HDL phospholipid fraction of the animals after exposure to smoke (26).

The more marked increase in the HDL core component, cholesteryl ester, demonstrated earlier (6) and verified in this study, suggests that a shift towards larger more

lipid-rich HDL₂ particles occurs after smoking withdrawal (6). Separation and quantitation of lipoprotein classes and HDL subclasses by zonal ultracentrifugation substantiates this interpretation, demonstrating more pronounced increases in the HDL₂ subfraction. This change in HDL₂/HDL₃ ratio is believed to be favorable from the atherogenic point of view (27). Similar changes in HDL subclasses are seen after endurance training programs (28). The mechanism for the increase in HDL levels remains speculative. A highly significant correlation between increase in fat intake and HDL cholesterol (Fig 4) as well as other HDL components (Table IV) indicate that nutritional factors contribute to the rise in HDL. In these normolipidemic subjects, where the triglyceride removal system, i.e. the lipoprotein lipase reaction, is working well below substrate saturation (29) an increased input of chylomicron triglyceride would automatically lead to an increased flux along the delipidation pathway without any accumulation of chylomicrons. Since the delipidation of chylomicrons is associated with transfer of chylomicron surface components, e.g. cholesterol, phospholipid, and apolipoprotein A, to the HDL fraction, an increased turnover of chylomicrons will also lead to an elevation of HDL levels (29). After transfer to HDL, part of the cholesterol will be transformed into cholesteryl ester by the LCAT system.

From fig 4 it seems that also the subjects who do not change their fat intake increased their HDL levels, on the average by about 0.1 mmol/l. About 50% of the rise in HDL, therefore, seems attributable to diet changes; this calculation is consistent with the coefficient of determination of 0.48 for change in dietary fat and HDL cholesterol, indicating that about 48% of the change in

HDL is explained by changes in fat intake.

The significant increase in postheparin LPL activities, after smoking cessation, together with significant correlations between changes in LPL activities and HDL components indicates that the rise in HDL, in addition to nutritional factors also is mediated via an increased activity of this enzyme. In parallel with an increased input of chylomicrons the rise in the activities of LPL and the other key enzyme in lipoprotein metabolism, LCAT, presumably will result in an enhanced flux along the delipidation pathway and hence in elevation of HDL concentrations.

The increase in cholesterol esterification rates was more pronounced when normal plasma was used as substrate (method of Glomset and Wright), which indicates that the increased esterification rate after smoking cessation was due to enhanced LCAT activity rather than changes in substrate quality. Further support for the view that the LCAT activity is affected by smoking is gained from the negative correlation between the cholesterol esterification rate and COHb levels found in the present study. Moreover, recently published data, show significantly lower LCAT levels in smokers compared with non-smokers (30), and it has also been demonstrated that cigarette smoke can depress LCAT activity (31). The differential effect of smoking cessation on cholesterol esterification measured with endogenous and exogenous substrate indicates that a change in substrate composition has occurred; the substrate quality after smoking cessation seems less favorable for the LCAT reaction. This difference can presumably be explained by the change in HDL_2/HDL_3 ratio, since HDL_2 has been reported to highly

inhibit the LCAT activity (32).

In summary, HDL levels rapidly and markedly increase after smoking cessation. Nutritional changes, especially increased intake of dietary fat, contribute to the elevated HDL levels. Alterations in the intravascular metabolism of lipoproteins, such as increased LPL activities, indicate that the rise in HDL concentration partly is mediated via this enzyme. Together the increased substrate availability (i e, chylomicron influx) and the enhanced enzyme activity would lead to an increased turnover of triglyceride-rich lipoproteins, which is known to be associated with increased HDL concentrations. The preferential rise in the HDL₂ subfraction is consistent with this interpretation.

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